Class	Count	E1>C1	E2>C2	**E3>C3**	E3>E1	E2>E1	E3>E2	E1<C1	E2<C2	**E3<C3**	E3<E1	E2<E1	E3<E2
BMP	11	0	18	9	9	0	0	0	0	0	0	0	0
Cer	35	0	40	31	31	40	6	0	0	9	9	0	20
DG	74	4	32	9	7	8	0	0	0	19	11	4	7
DMPE	13	0	15	23	15	23	0	0	23	23	23	15	8
ether-PC	29	0	3	0	7	10	0	7	24	41	7	0	10
ether-PE	22	0	9	5	14	32	0	0	27	32	5	0	14
ether-PS	10	0	0	0	40	20	30	0	0	10	0	0	0
ether-TG	46	0	20	0	4	50	0	2	20	48	13	9	37
HexCer	6	0	0	0	0	0	0	33	33	33	33	0	17
LPC	22	0	0	0	0	0	0	0	64	77	86	41	64
LPE	10	10	10	10	0	0	0	0	30	40	50	20	20
OxLPC	8	0	0	0	0	0	0	0	0	13	50	0	38
OxPC	9	0	56	44	11	11	0	0	0	0	11	0	11
OxPE	8	0	38	63	0	0	0	0	0	0	0	0	0
OxTG	89	12	93	91	89	26	19	1	2	3	1	0	0
PC	98	0	3	6	4	8	1	2	52	61	60	37	43
PE	72	4	18	21	22	18	6	3	32	36	26	22	10
PG	23	4	30	22	48	61	9	0	0	13	4	0	4
PI	21	0	0	0	5	0	0	0	24	5	5	5	5
PS	9	0	22	33	0	44	0	0	0	0	33	0	89
SM	23	4	9	30	26	26	0	0	9	48	48	0	48
TG	285	1	85	33	60	66	0	0	0	0	0	0	0

Table S1A: Percent of lipids which decreased or increased across time in the liver of ethanol-fed mice (2 wks (E1), 4 wks (E2), and 5 wks (E3)) and in comparison to controls (C1, C2, and C3); for example E1>C1 is the percent of lipids which are greater in the 2nd week ethanol fed mice as compared to controls at the 2nd week. A lipid species was considered to increase or decrease when $p < 0.05$ for an FDR corrected (Hochberg) ANOVA with a Tukey's *post hoc* test, and if the species changed by over 25%. The values from this table were used to generate Figure 2.

Class	Count	E1>C1	E2>C2	**E3>C3**	E3>E1	E2>E1	E3>E2	E1<C1	E2<C2	**E3<C3**	E3<E1	E2<E1	E3<E2
AcCar	17	6	59	53	29	47	0	0	0	0	0	0	0
CE	8	13	0	0	0	0	0	0	13	13	50	75	0
Cer	14	0	0	0	0	0	0	0	21	36	43	43	0
DG	9	33	0	0	0	0	0	0	22	22	89	78	0
DMPE	6	0	0	0	0	0	0	0	17	0	83	83	0
ether-LPC	6	17	0	0	0	0	0	0	0	0	83	83	0
ether-PC	31	6	0	3	0	0	0	0	3	3	10	19	0
ether-PE	17	6	0	0	0	0	0	0	0	0	6	6	0
ether-TG	41	0	0	0	0	0	0	0	0	78	49	0	20
HexCer	6	0	0	0	0	0	0	0	33	33	33	50	0
LPC	25	0	0	0	0	0	0	0	40	44	80	100	0
LPE	7	29	0	0	0	0	0	0	14	14	43	86	0
OxLPC	44	0	0	0	0	0	0	0	0	7	7	0	5
OxPC	14	0	0	0	0	0	0	0	0	0	0	0	0
OxTG	18	6	11	6	0	0	0	0	0	0	0	0	0
PC	78	3	0	1	0	0	0	0	54	44	73	79	0
PE	12	0	0	0	0	0	0	0	42	58	92	67	8
PI	12	0	0	8	8	8	0	0	0	0	0	0	0
SM	21	90	0	0	0	0	0	0	0	10	48	57	0
TG	147	11	1	2	0	0	0	0	8	7	41	40	1

Table S1B: Same as Table S1A, but for plasma lipids.

Weekly Average Intake per Mouse (kilocalories)

Weeks of Study	Group 1		Group 2		Group 3	
	Ethanol Diet (GE1)	Control Diet (GC1)	Ethanol Diet (GE2)	Control Diet (GC2)	Ethanol Diet (GE3)	Control Diet (GC3)
Week 1 (2% v/v EtOH)	74.61 ± 4.47	80.33 ± 5.97	77.15 ± 5.44	70.3 ± 5.37	80.14 ± 6.33	75.46 ± 4.88
Week 2 (3% v/v EtOH)	74.33 ± 4.68	82.00 ± 7.20	69.5 ± 5.93	67.33 ± 7.02	69.43 ± 3.63	74.21 ± 7.99
Week 3 (4% v/v EtOH)			75.2 ± 5.90	81.33 ± 4.23	74.71 ± 3.34	79.64 ± 4.84
Week 4 (5% v/v EtOH)			70.8 ± 10.03	80.83 ± 5.52	64.0 ± 3.45	77.21 ± 6.43
Week 5 (6% v/v EtOH)					69.5 ± 13.01	84.5 ± 10.39

Table S2: Recorded kilocalorie intake for mice across all groups and time points (average and standard deviation). No comparisons were significant using Hochberg correction between ethanol-fed and control mice.

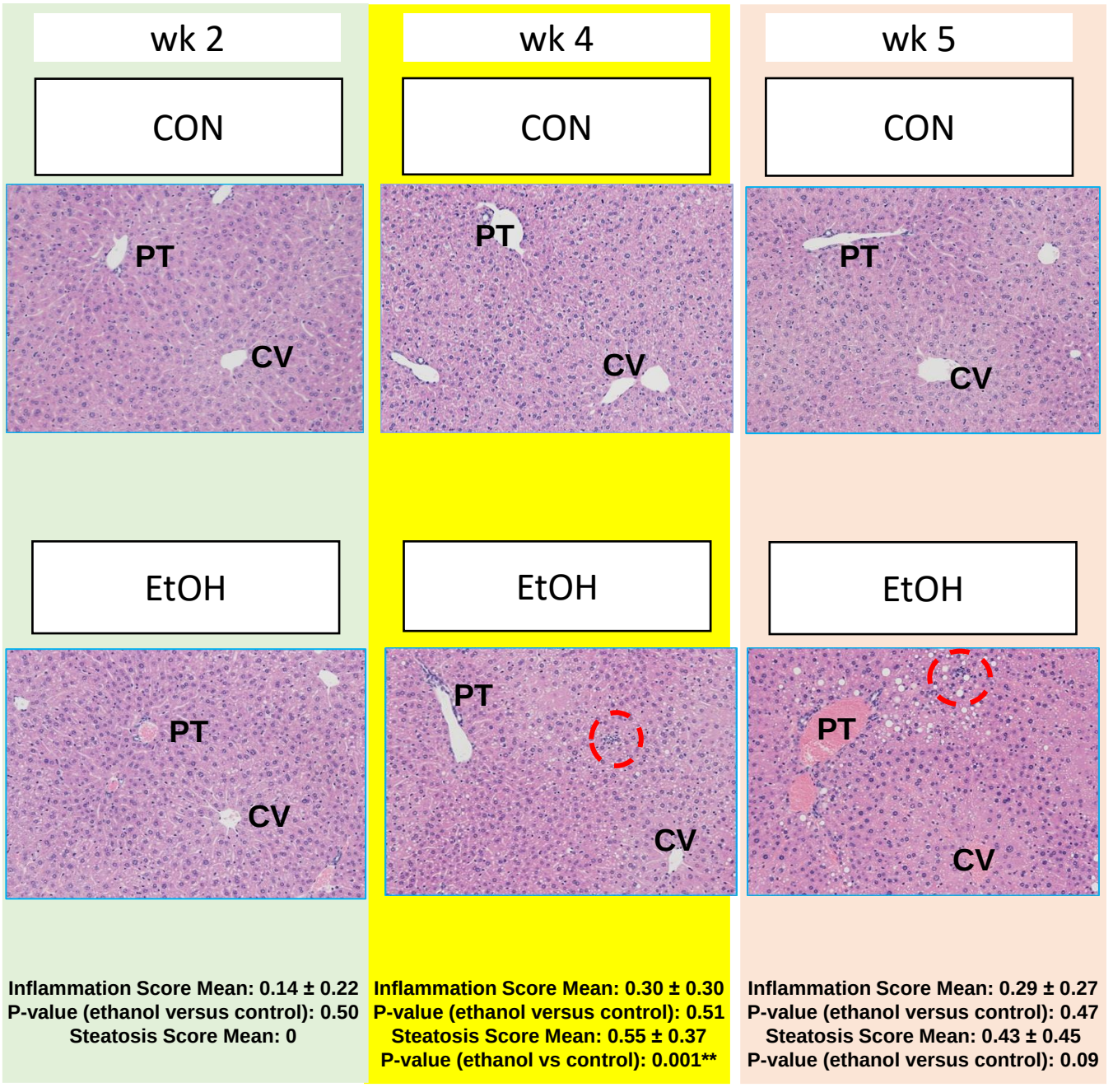


Figure S1: Representative histological images of liver after different durations of ethanol consumption. Mice were fed ethanol (EtOH) or a control carbohydrate (CON,) diet for 5 weeks. Groups of mice were euthanized at the end of the second (wk 2), fourth (wk4) and fifth (wk 5) week of EtOH or carbohydrate feeding. Dashed red circles indicate small inflammatory foci. Magnification: 200x. Scores were determined by DJO according to the following reference: Lanaspa, M. A. et al. Ketohexokinase C blockade ameliorates fructose-induced metabolic dysfunction in fructose-sensitive mice. J. Clin. Invest. 128, 2226–2238 (2018).

Score averages, standard deviations, and p-values (Student t-test, unequal variance, two sided) are shown in the bottom of panels and the raw data for which these values were derived is provided in a supplemental excel.

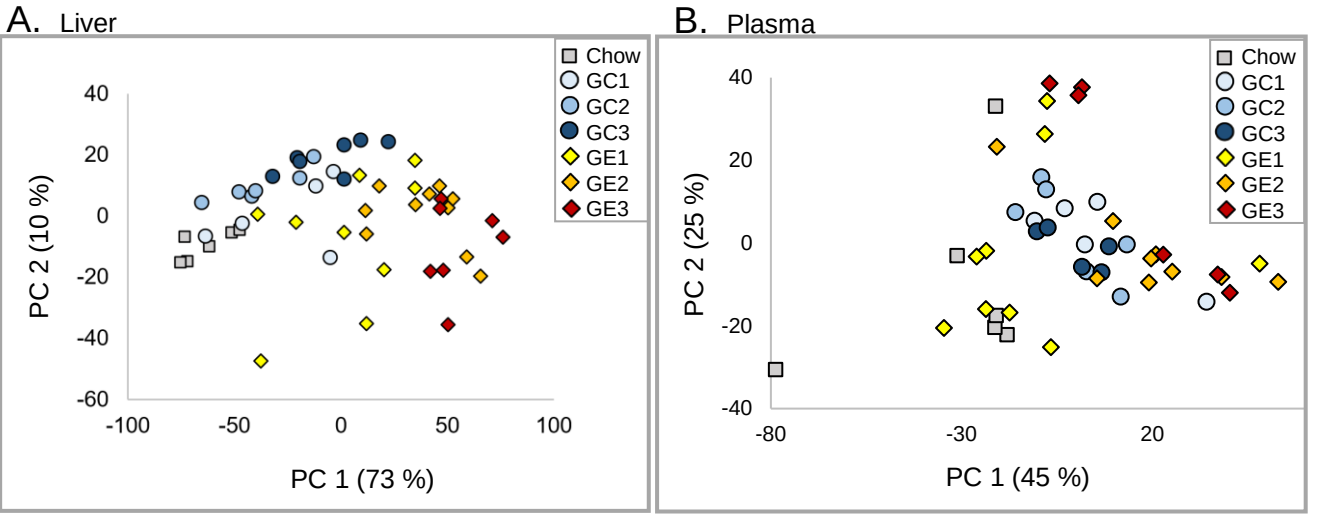


Figure S2: Principal component analysis (PCA) of liver (A) and plasma (B) samples from mice fed standard chow (chow), carbohydrates (GC) or ethanol (GE) for 2 (GC1, GE1), four (GC2, GE2) or five (GC3, GE3) weeks. Principal components are derived from lipidomics data after filtering, annotating, and combining negative and positive polarity using LipidMatch Flow.

The following comparisons were made using multiple t-tests and Hochberg correction:
Chow vs GE1, GC1 vs GE1, GC2 vs GE2, GC3 vs GE3, GE1 vs GE2, GE2 vs GE3, and GE1 vs GE3.
No comparisons between groups were significant for mouse plasma except between GE1 and GE2, for mouse liver the following were significant (<0.05 FDR corrected p-values using Benjamini-Hochberg):

Comparison	Benjamini-Hochberg Adjusted p-value
Chow vs GE1	0.00071
GC2 vs GE2	0.00003
GC3 vs GE3	0.00014
GE1 vs GE3	0.00160
GE1 vs GE2	0.00910

Selected Box Plots of Lipids in Liver

Note for Figure S3A-S3S and Figure S4A-S4E: All “original concentration” values were normalized to a single class-based internal standard and therefore cannot be considered quantitative, but rather semi-quantitative. Units are µg lipid / g sample (liver or plasma). The “normalized values” are mean centered and log transformed. The boxplots show minimum, first, second and third quartile, and maximum. Dots represent individual samples.

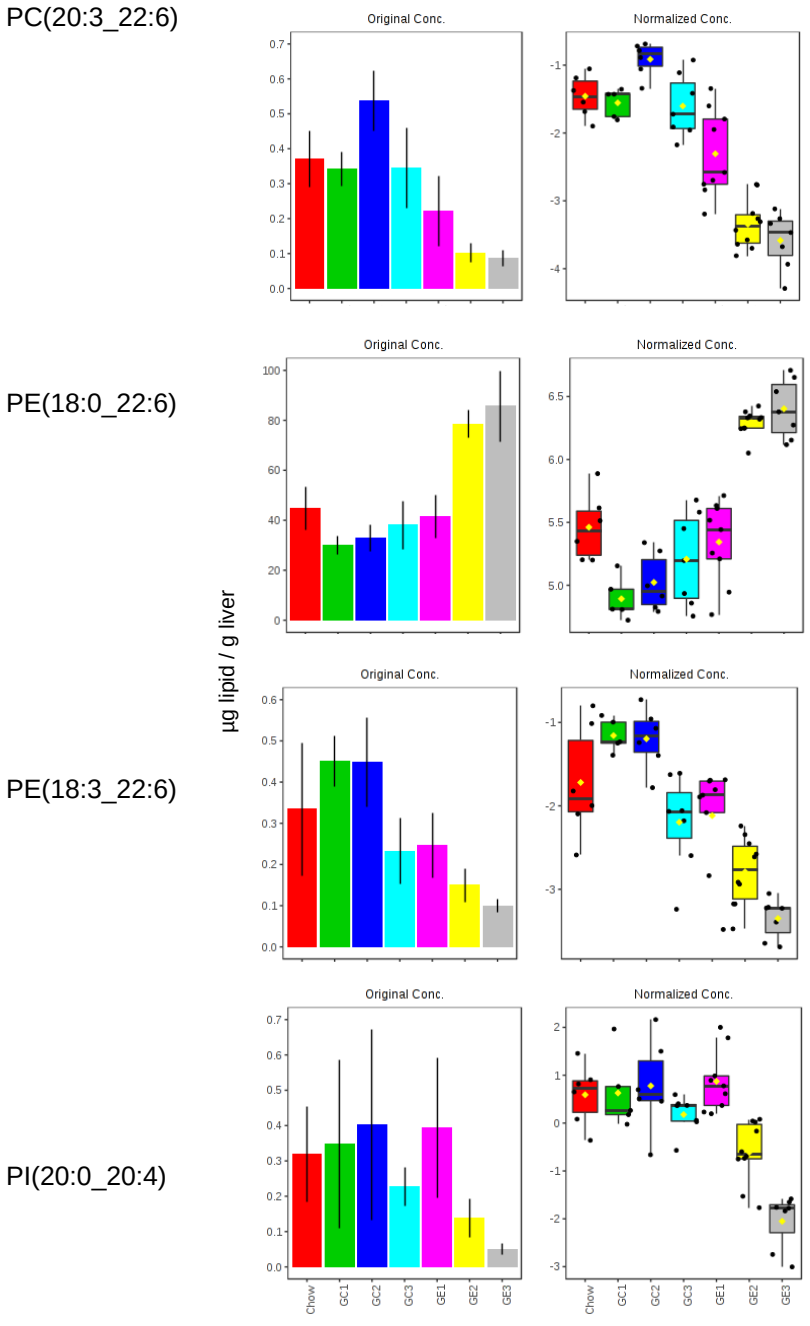


Figure S3A: Glycerophospholipids. GE: ethanol-fed; GC: pair-fed control; PE: phosphatidylethanolamine; PI: phosphatidylinisitiol. On the left original concentrations are normalized to class based internal standards (semi-quantitative; µg/g), and on the right normalized concentrations represent log transformed and mean centered data. All species shown except PI are significantly different (normalized data) between the 2nd and 5th week of ethanol treatment, and between all time points and pair-fed controls according to an FDR corrected (Hochberg) ANOVA with a Tukey *post-hoc* test (p-value < 0.05). PI was significantly different between the 2nd and 5th week of ethanol-fed, and between the 4th and 5th week of ethanol-fed and respective pair-fed controls.

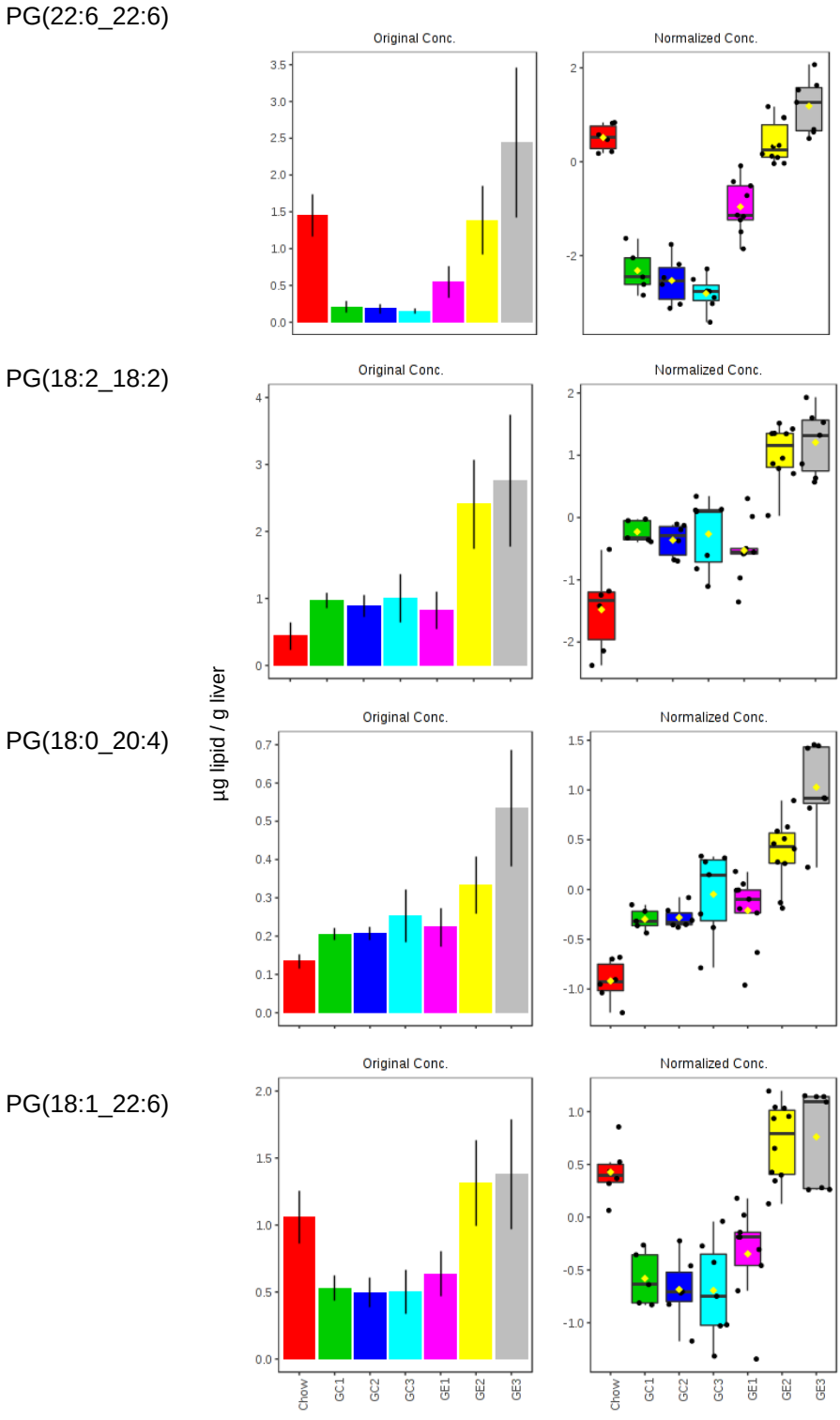


Figure S3B: Phosphatidylglycerol. PG(22:6/22:6) is significantly different the 2nd and 5th week of ethanol treatment, and between all time points and controls according to an FDR corrected (Hochberg) ANOVA with a Tukey *post-hoc* test (p-value < 0.05). The remaining PG were significantly different between the 4th and 5th week and respective controls, and between the 2nd and 5th week of alcohol feeding. SEE FIG. S3A COMMENTS

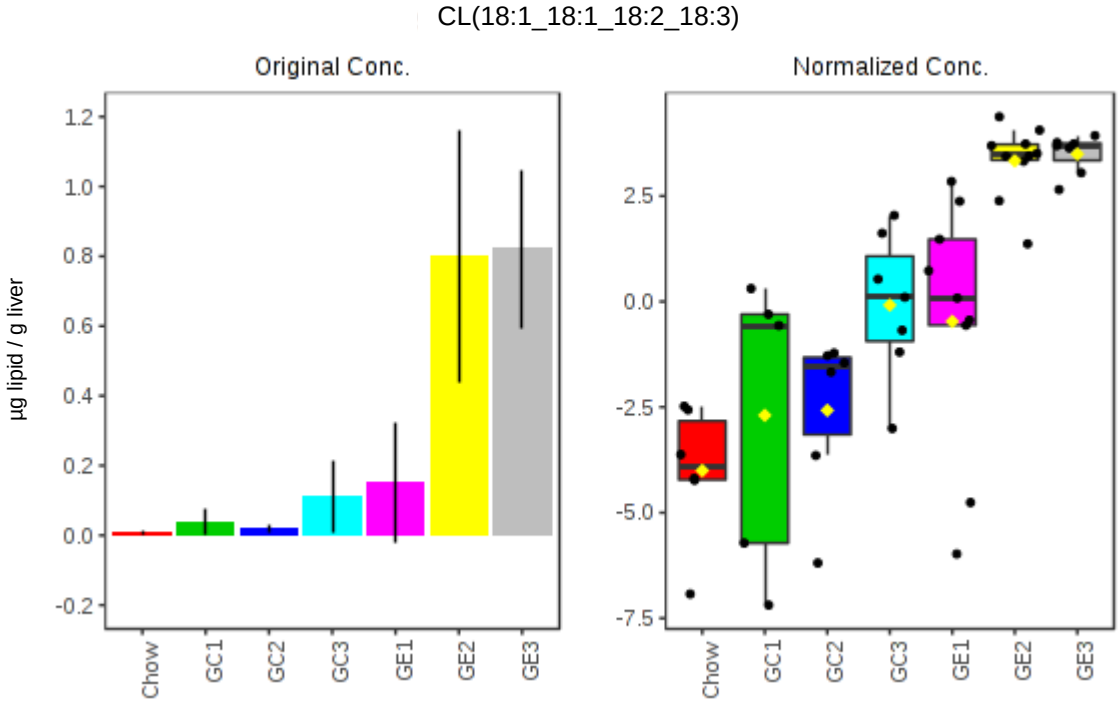


Figure S3C: Cardiolipin (CL(18:1_18:1_18:2_18:3)) was significantly different between all time points and controls, and between the first and last time point. SEE FIG. S3A COMMENTS

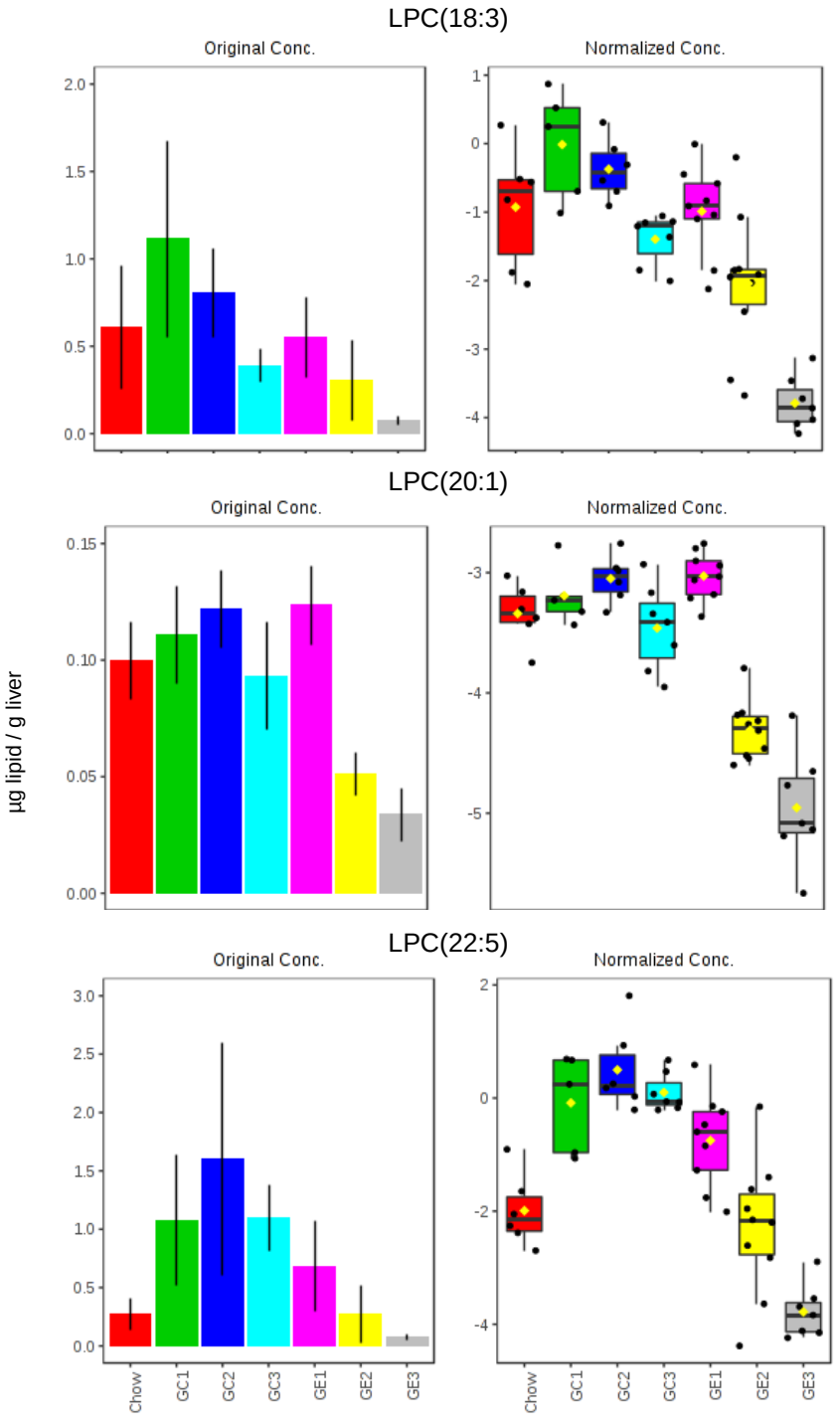


Figure S3D: Glycerophospholipids. All species shown are significantly different between 4 weeks and 5 weeks (ethanol treated), and between 4 weeks and 5 weeks and the respective controls. LPC=lysophosphatidylcholine. SEE FIG. S3A COMMENTS

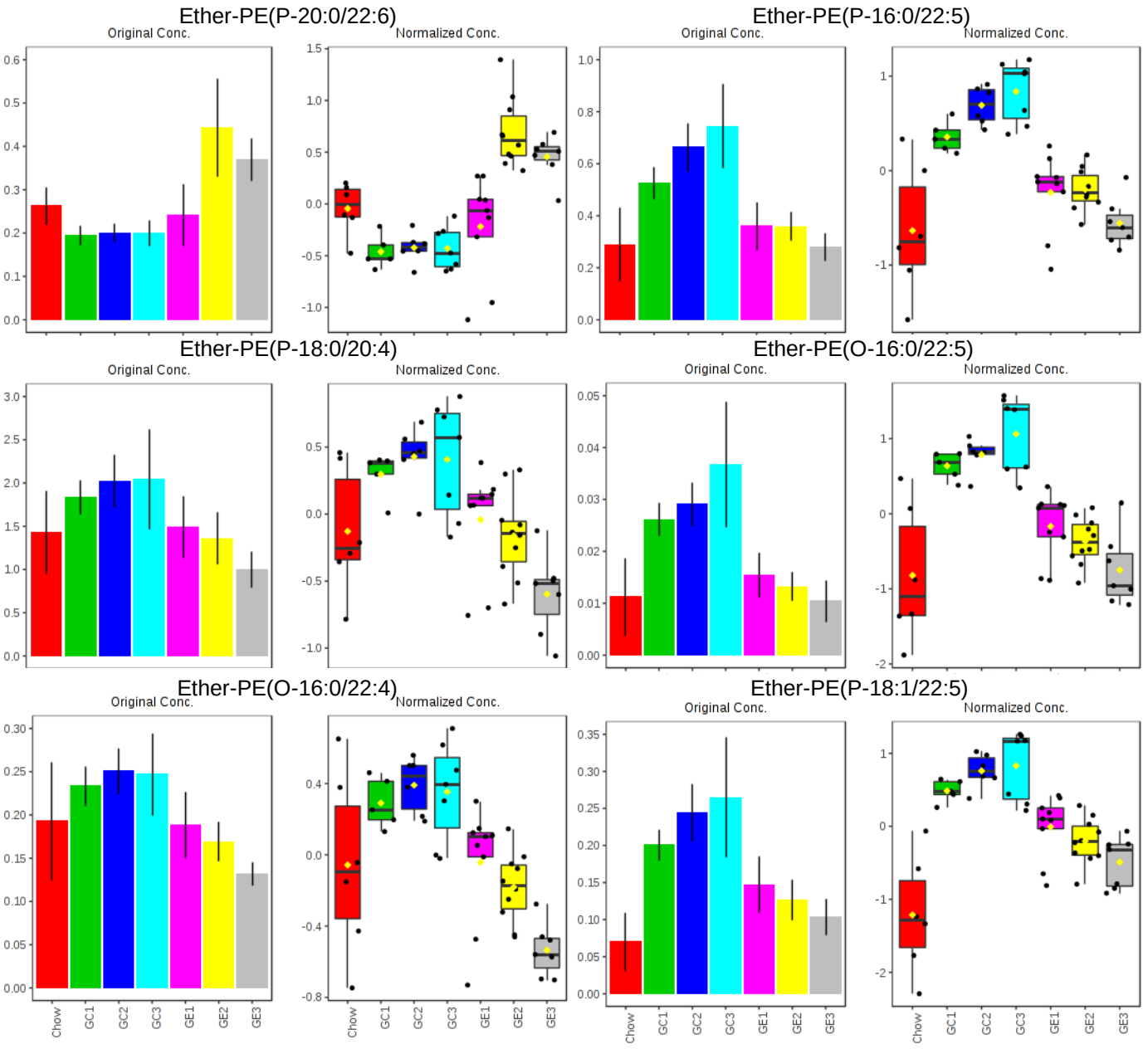


Figure S3E: ether-glycerophospholipids. PE: phosphatidylethanolamine; PC: phosphatidylcholine; O-: ether; P-: vinyl ether. All species shown are significantly different between 4 weeks and 5 weeks (ethanol treated), and between 4 weeks and 5 weeks and the respective controls. Original concentrations are in µg lipid / g liver. SEE FIG. S3A COMMENTS

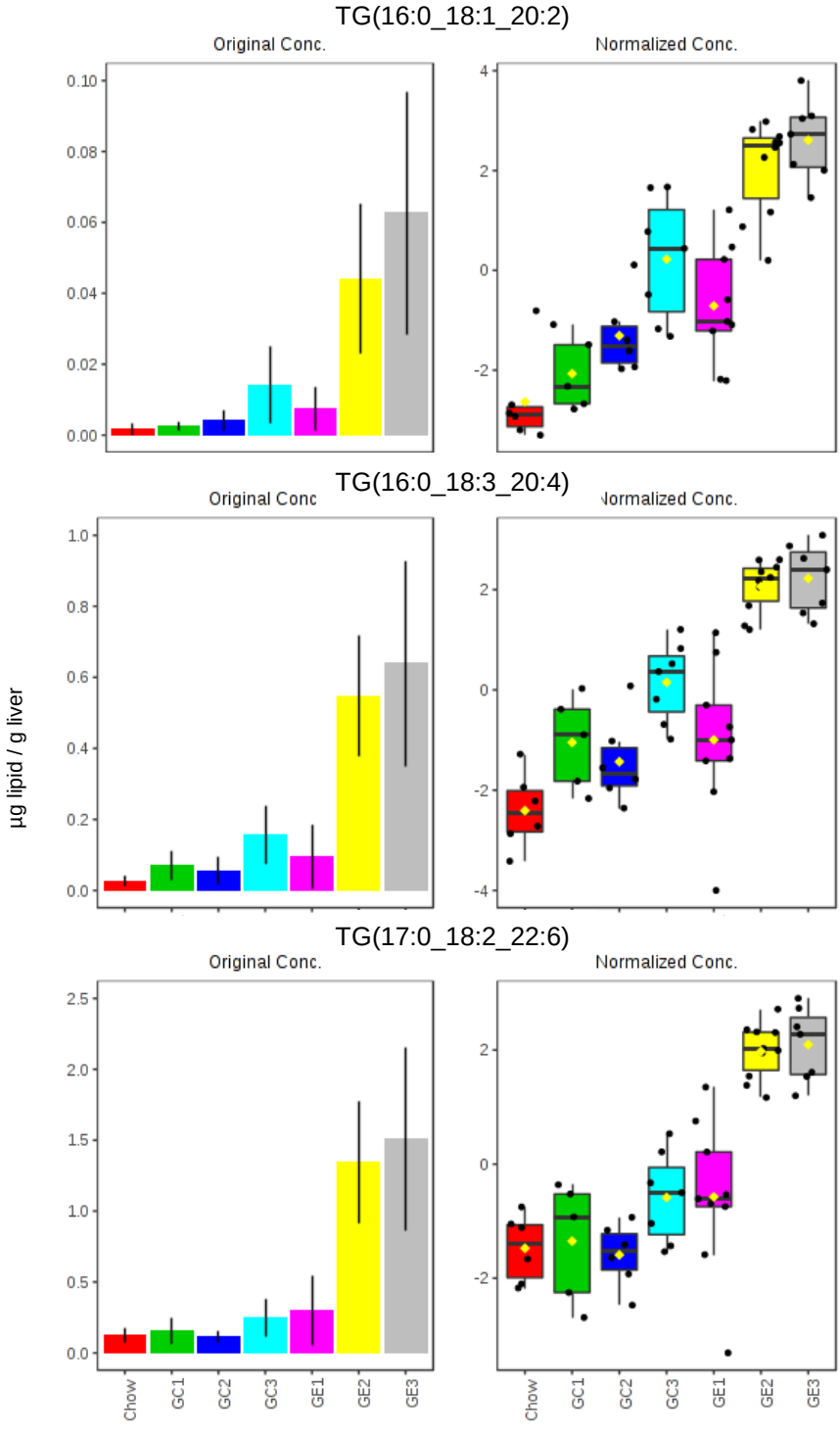


Figure S3F: Triglycerides (TG). All species shown are significantly different between 4 weeks and 5 weeks (ethanol treated), and between 4 weeks and 5 weeks and the respective controls. SEE FIG. S3A COMMENTS

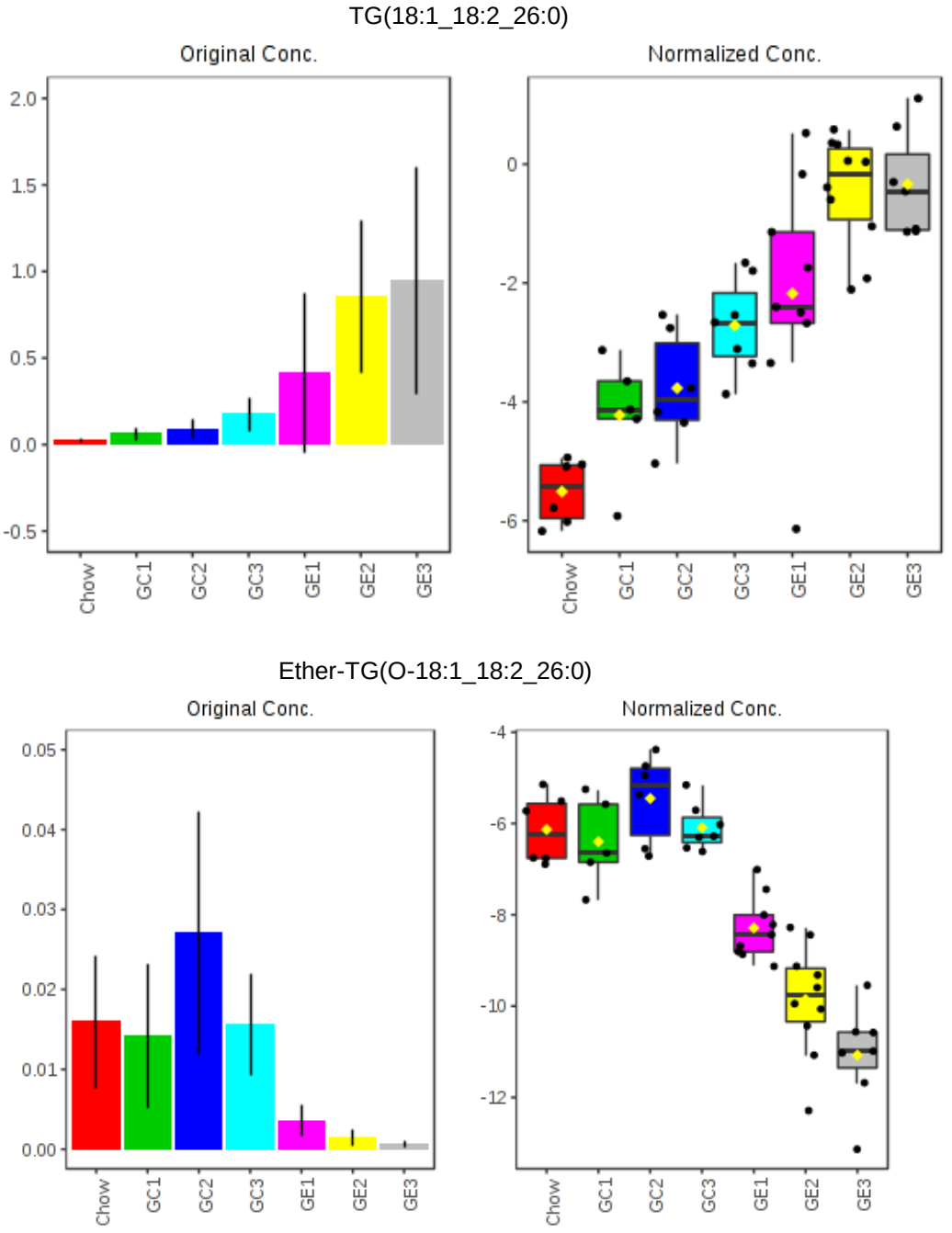


Figure S3G: Triglycerides (TG) and ether-triglycerides. All species shown are significantly different across all time points for ethanol treatment, and between all time points and controls. SEE FIG. S3A and S3E COMMENTS

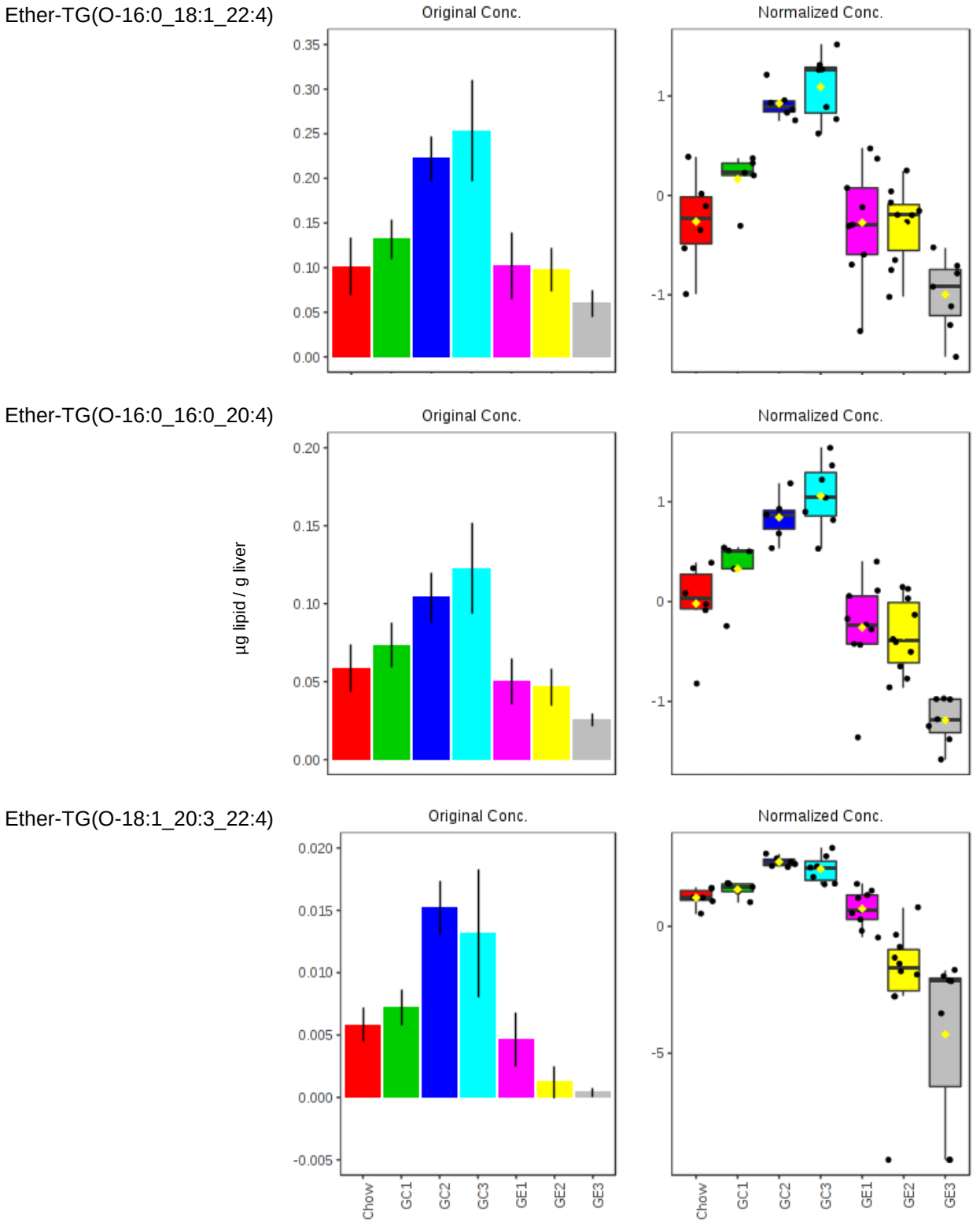


Figure S3H: Ether-triglycerides (Ether-TG). All species shown are significantly different between 4 weeks and 5 weeks (ethanol treated), and between 4 weeks and 5 weeks and the respective controls. SEE FIG. S3A and S3E COMMENTS

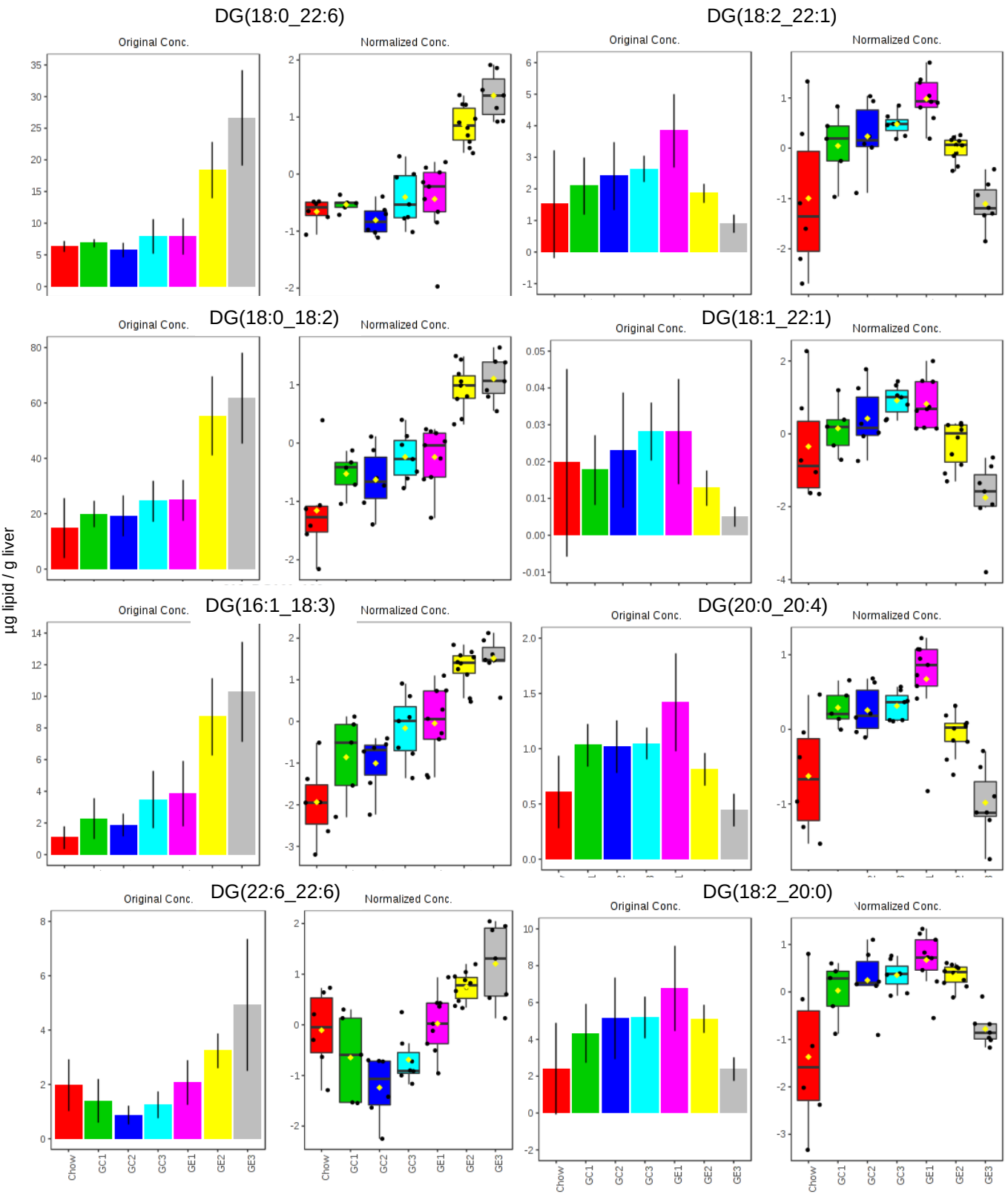


Figure S3I: All diglyceride (DG) species shown are significantly different between 4 weeks and 5 weeks (ethanol treated), and between 4 weeks and 5 weeks and the respective controls. SEE FIG. S3A COMMENTS

OxTG(18:1_18:2_18:2(OH))

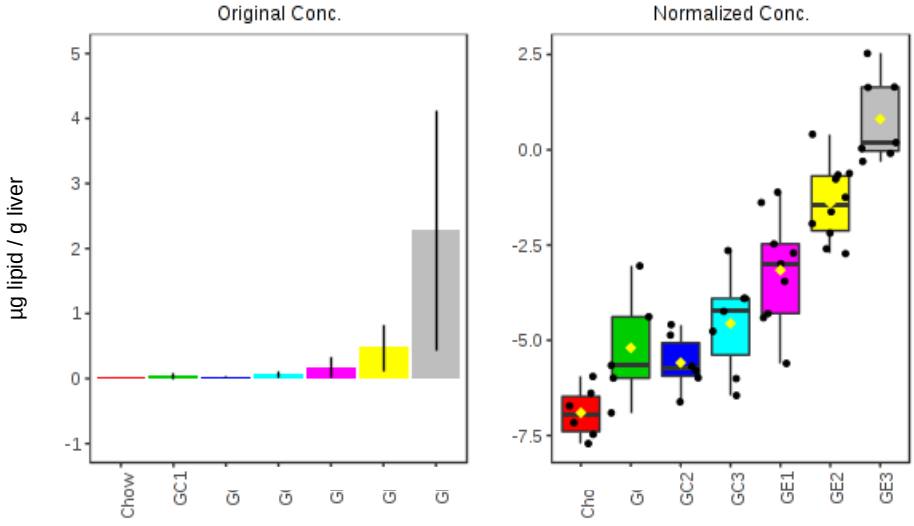
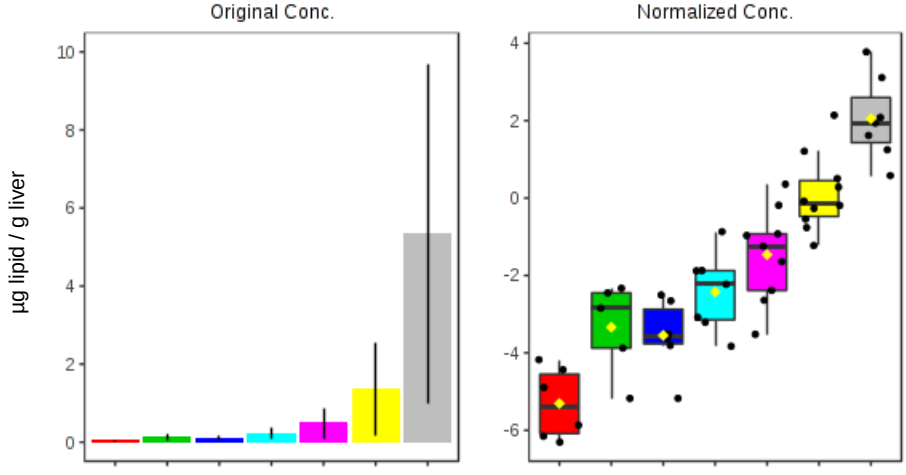


Figure S3J: Hydroxyl (OH) containing oxidized triglyceride (OxTG). All species shown are significantly different between the 2nd and 5th week, and between all time points and controls. SEE FIG. 3A COMMENTS

OxTG(18:1_18:2_18:2(Ke_or_Epoxy))



OxTG(16:0_18:2_18:2(Ke_or_Epoxy))

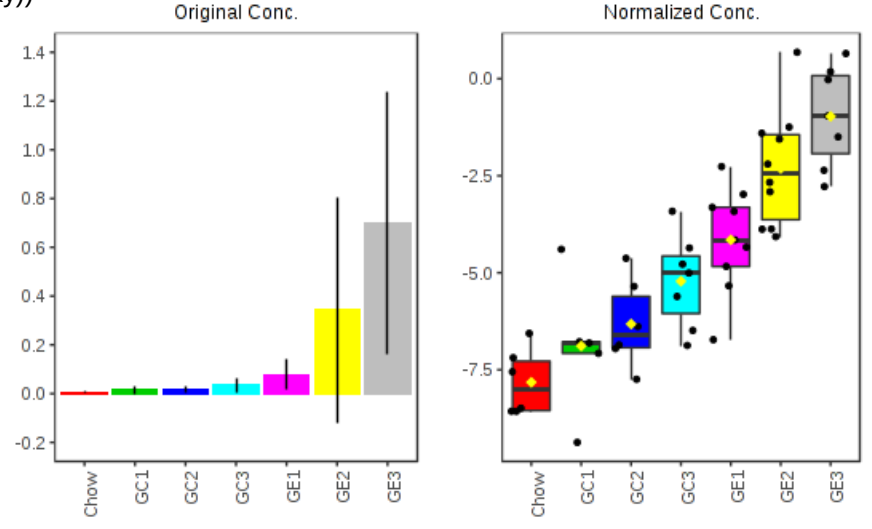
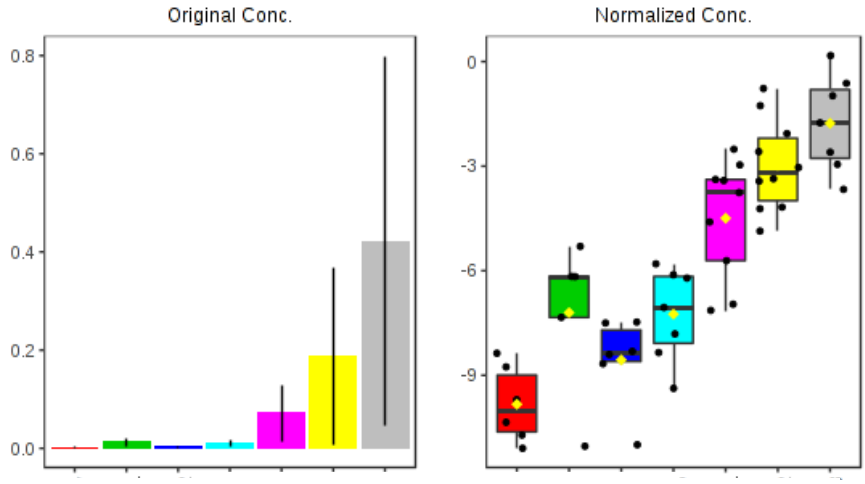


Figure S3K: Ketone or Epoxy containing oxidized triglyceride (OxTG). All species shown are significantly different between the 2nd and 5th week, and between all time points and controls. SEE FIG. 3A COMMENTS

OxTG(18:1_18:2_9:0(CHO))



OxTG(18:1_18:2_4:0(CHO))

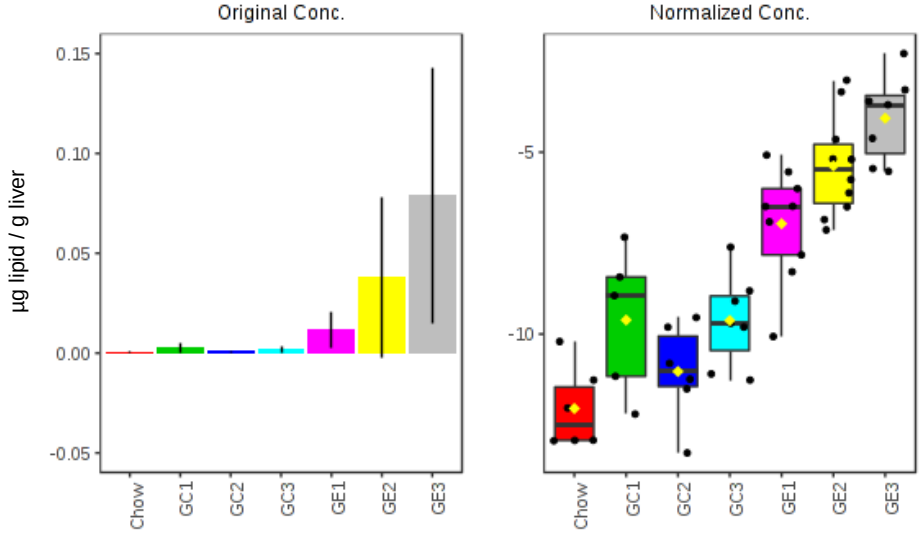


Figure S3L: Short chain oxidized triglycerides (OxTG) contain aldehyde (CHO). All species shown are significantly different between the 2nd and 5th week, and between all time points and controls. SEE FIG. S3A COMMENTS

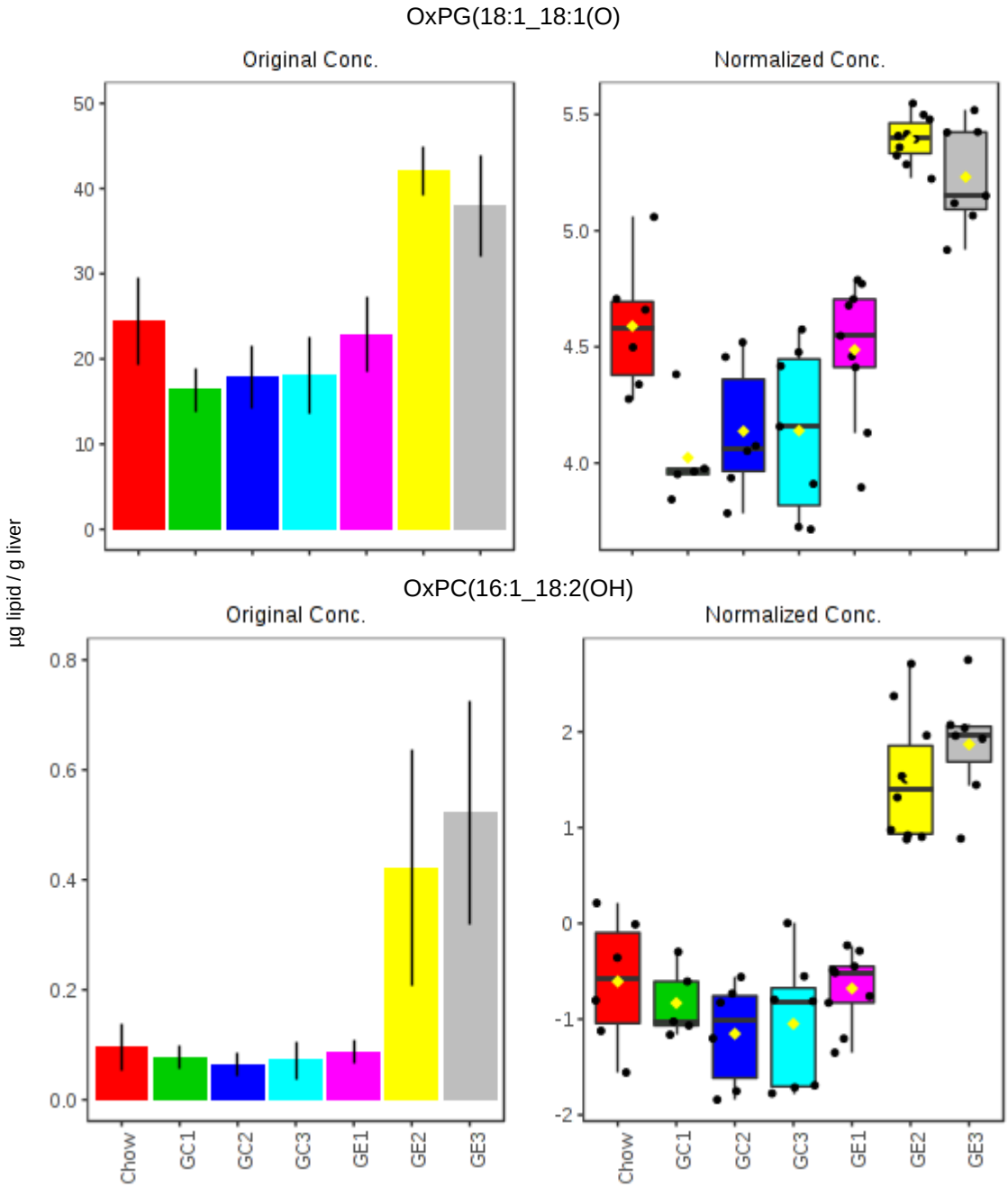


Figure S3M: Oxidized glycerophospholipids. Species shown are significantly different between the first and last time point, and all time points and controls. Abbreviations as follows OxPG=oxidized phosphatidylglycerol, OxPC=oxidized phosphatidylcholine. SEE FIG. S3A COMMENTS

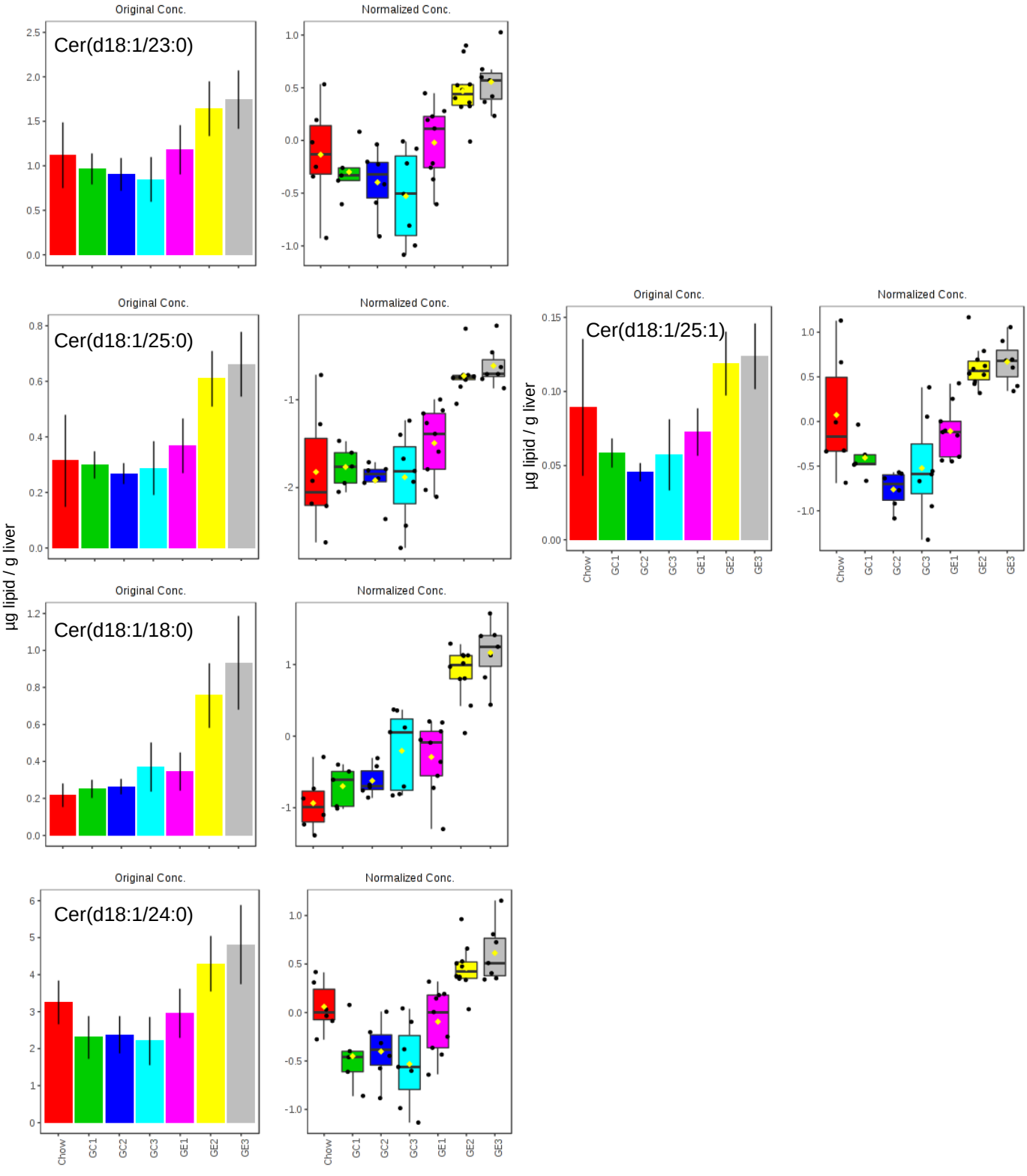


Figure S3N: Ceramides. Ceramides shown are significantly different between the first and last time point, and all time points and controls. SEE FIG. S3A COMMENTS

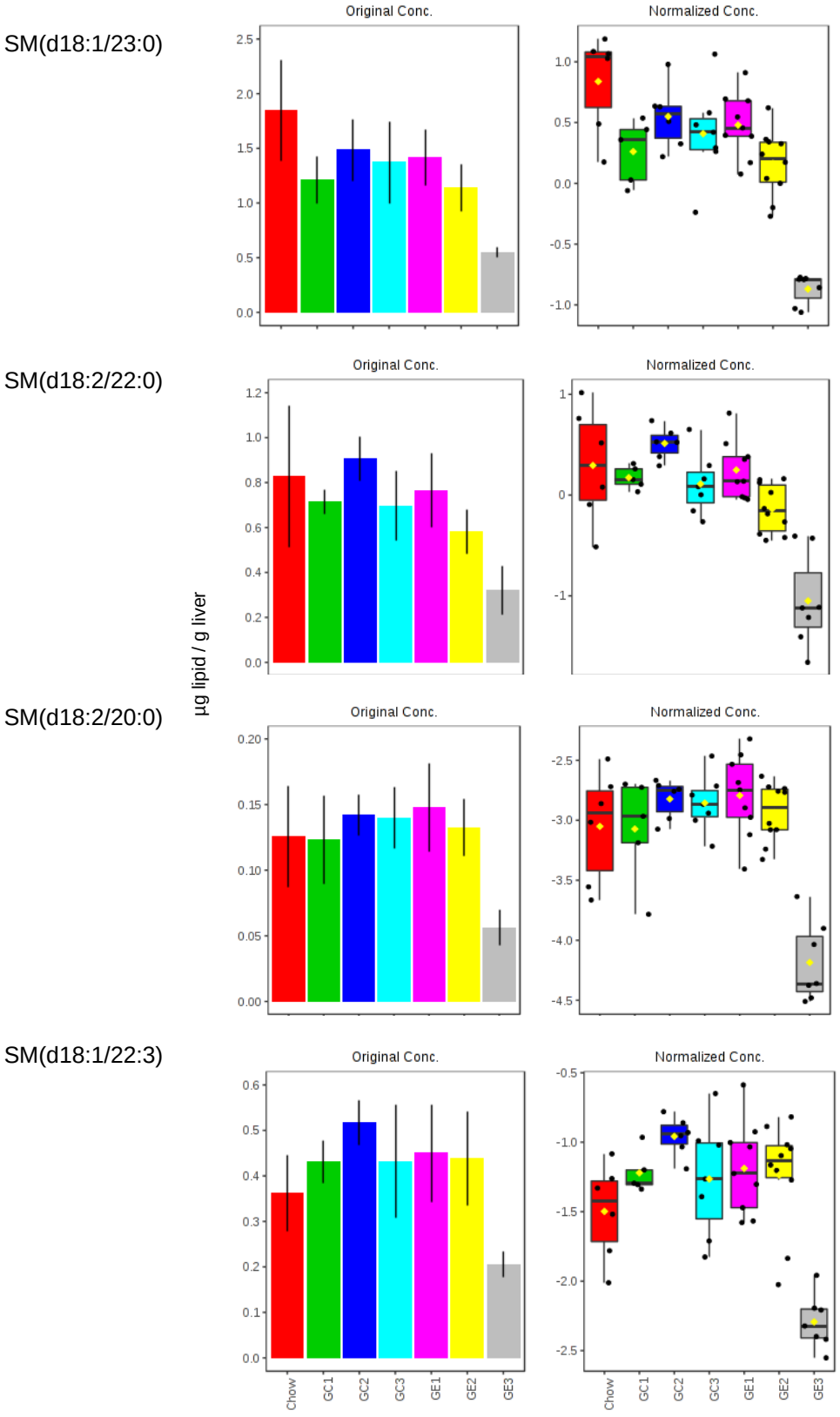


Figure S3O: Sphingomyelins shown are significantly different in week 5 versus 4, and in respective controls for week 5. SEE FIG. S3A COMMENTS

SM(d17:1/18:0)

SM(d18:1/18:0)

SM(d18:1/18:3)

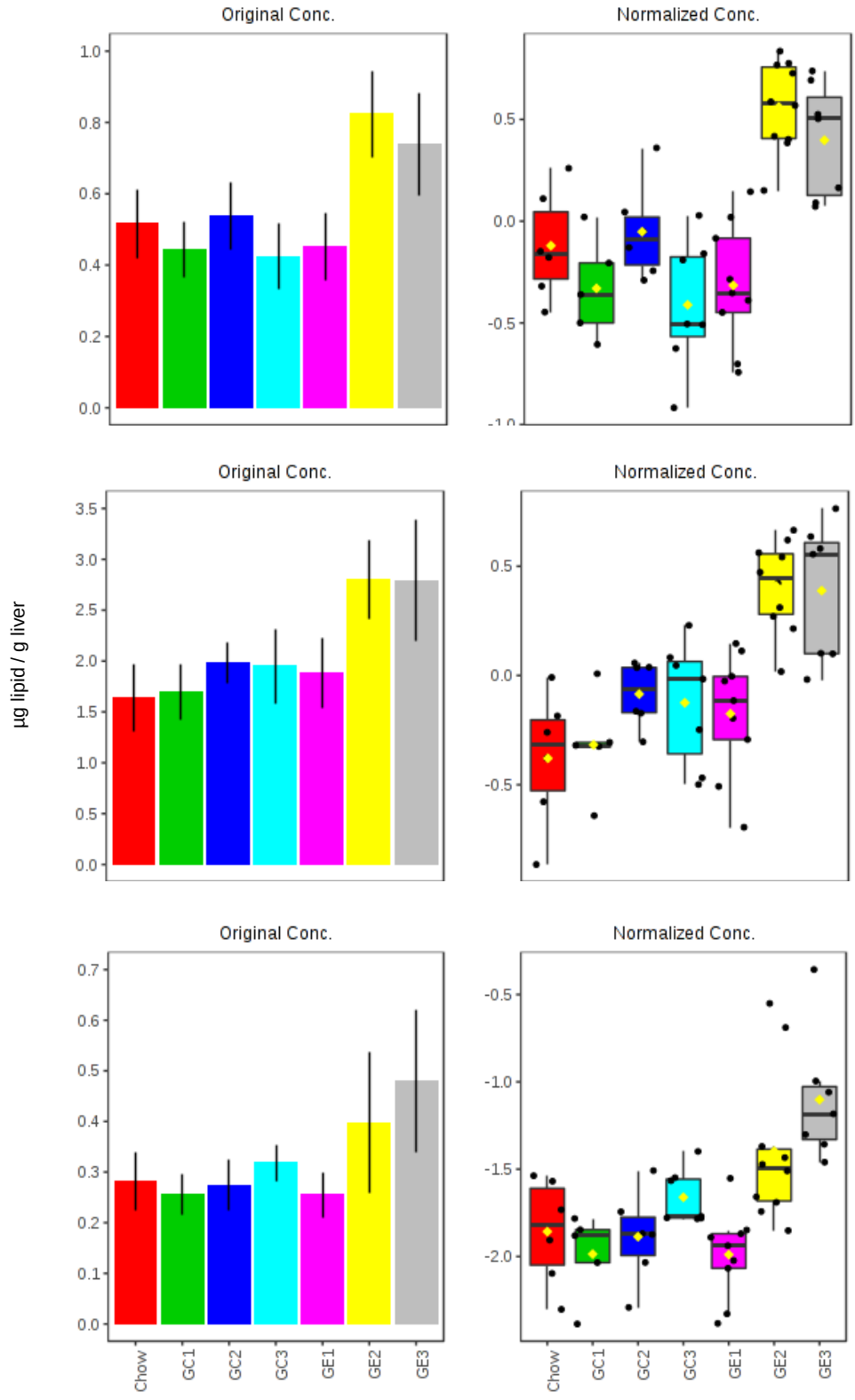


Figure S3P: Sphingomyelins shown are significantly different in week 5 versus 4, and in respective controls for week 5. SEE FIG. S3A COMMENTS

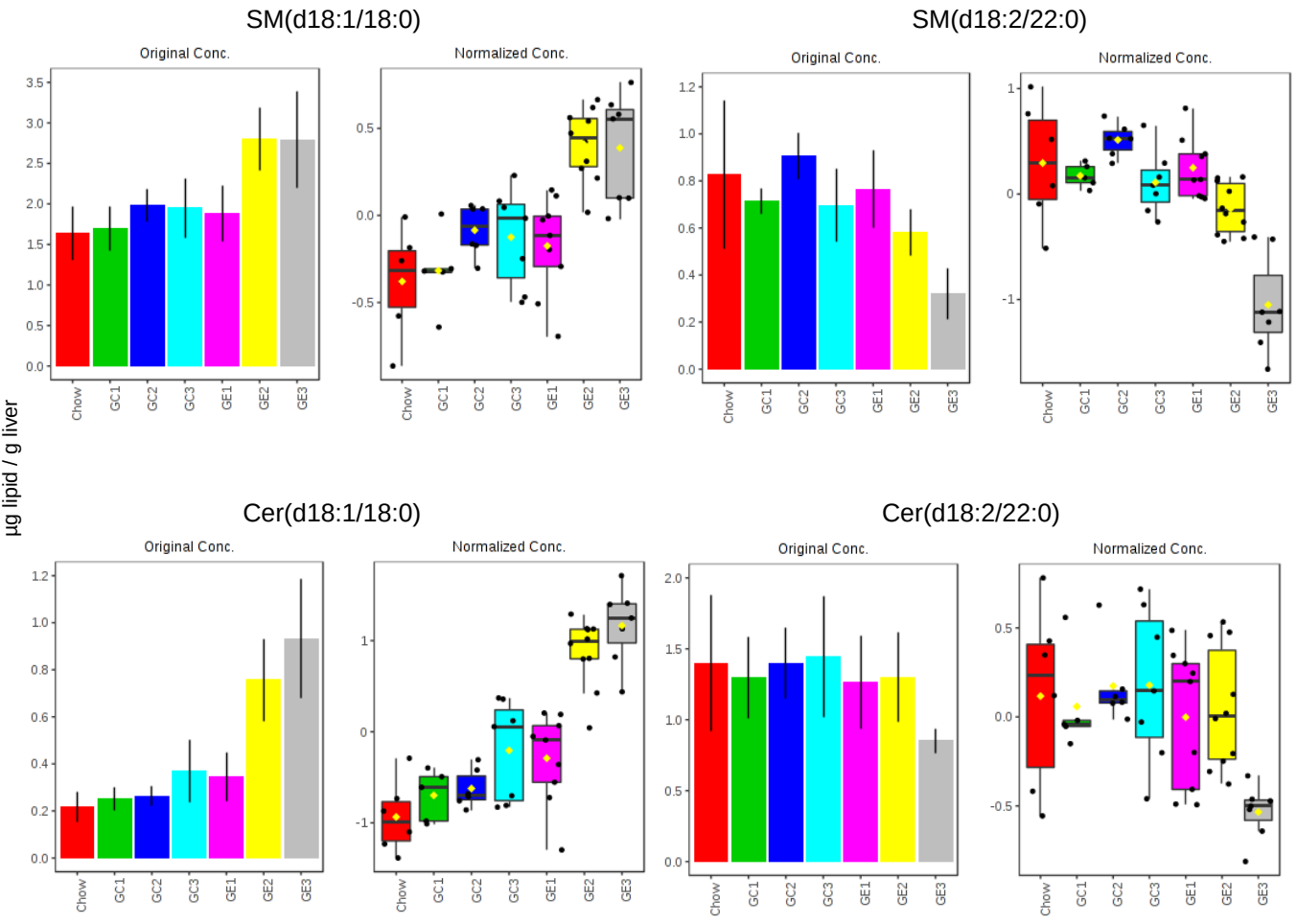


Figure S3Q: Sphingomyelins and ceramides shown are significantly different in week 5 as compared to controls and week 1 and classes with corresponding fatty acyl chains correlate. SEE FIG. S3A COMMENTS

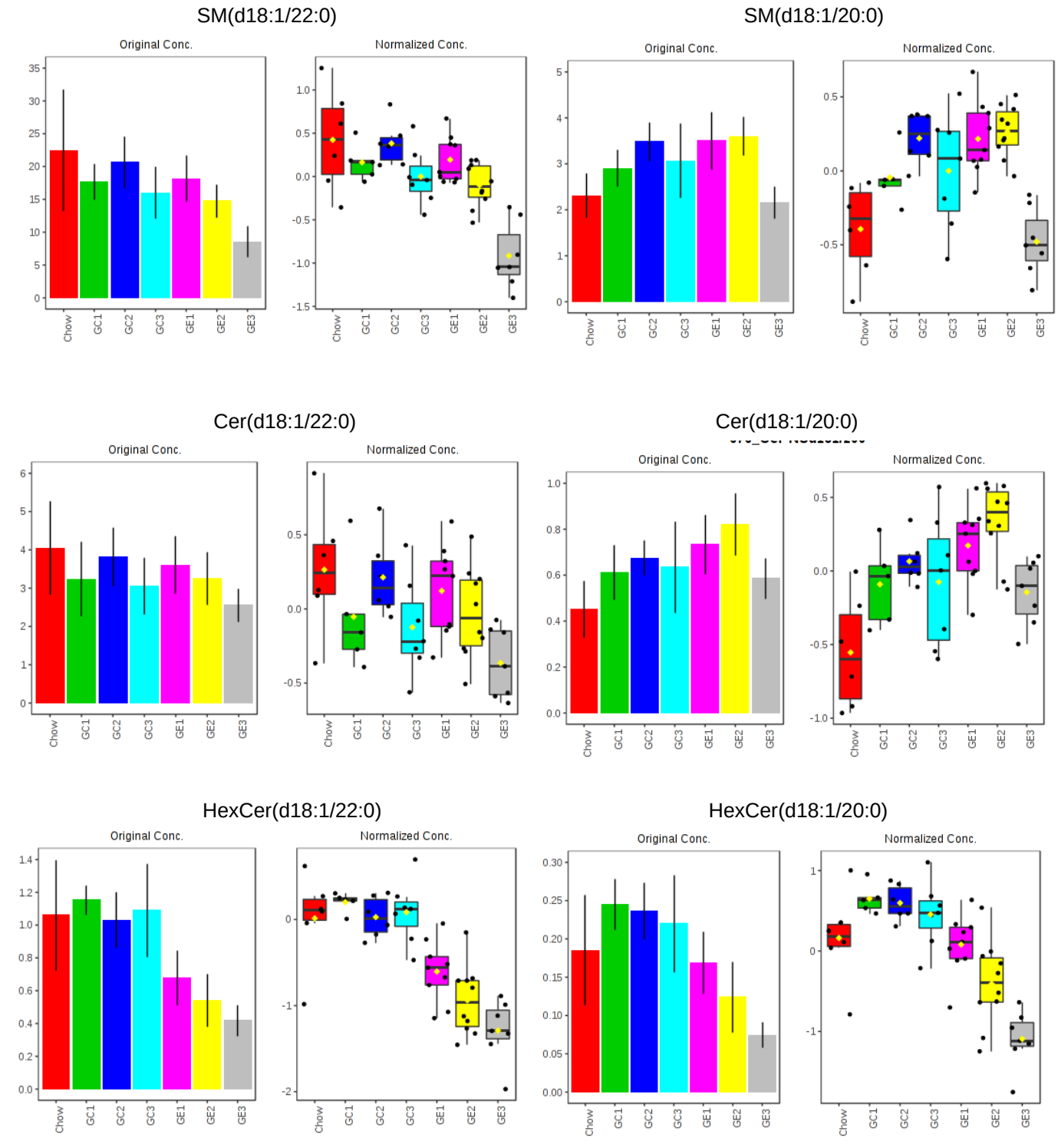


Figure S3R: Sphingomyelins, ceramides and hexosylceramides shown are significantly different in week 5 as compared to controls and week 1 and classes with corresponding fatty acyl chains correlate, although correlation is weak between hexosylceramides and other corresponding sphingolipids. SEE FIG. S3A COMMENTS

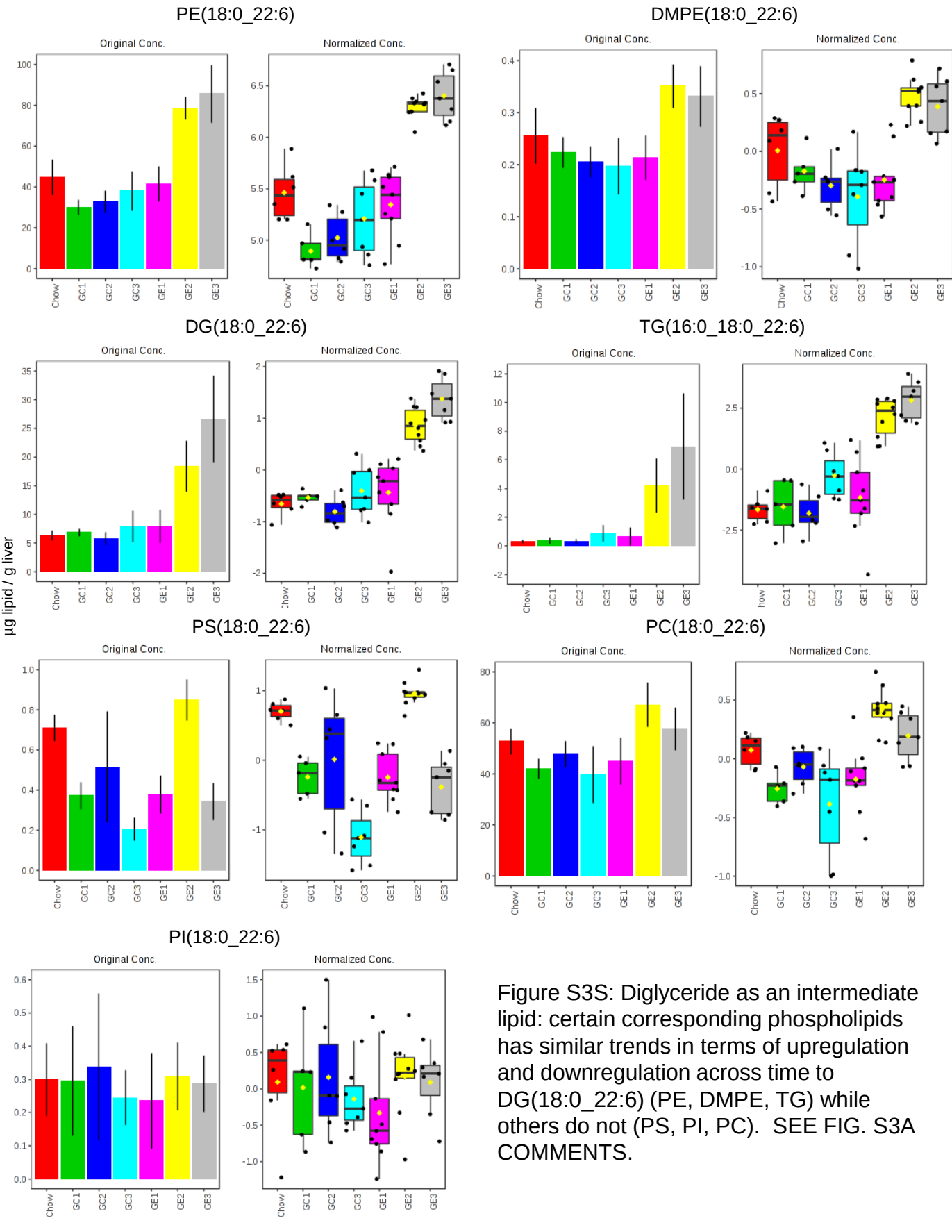


Figure S3S: Diglyceride as an intermediate lipid: certain corresponding phospholipids has similar trends in terms of upregulation and downregulation across time to DG(18:0_22:6) (PE, DMPE, TG) while others do not (PS, PI, PC). SEE FIG. S3A COMMENTS.

Selected Box Plots of Lipids in Plasma

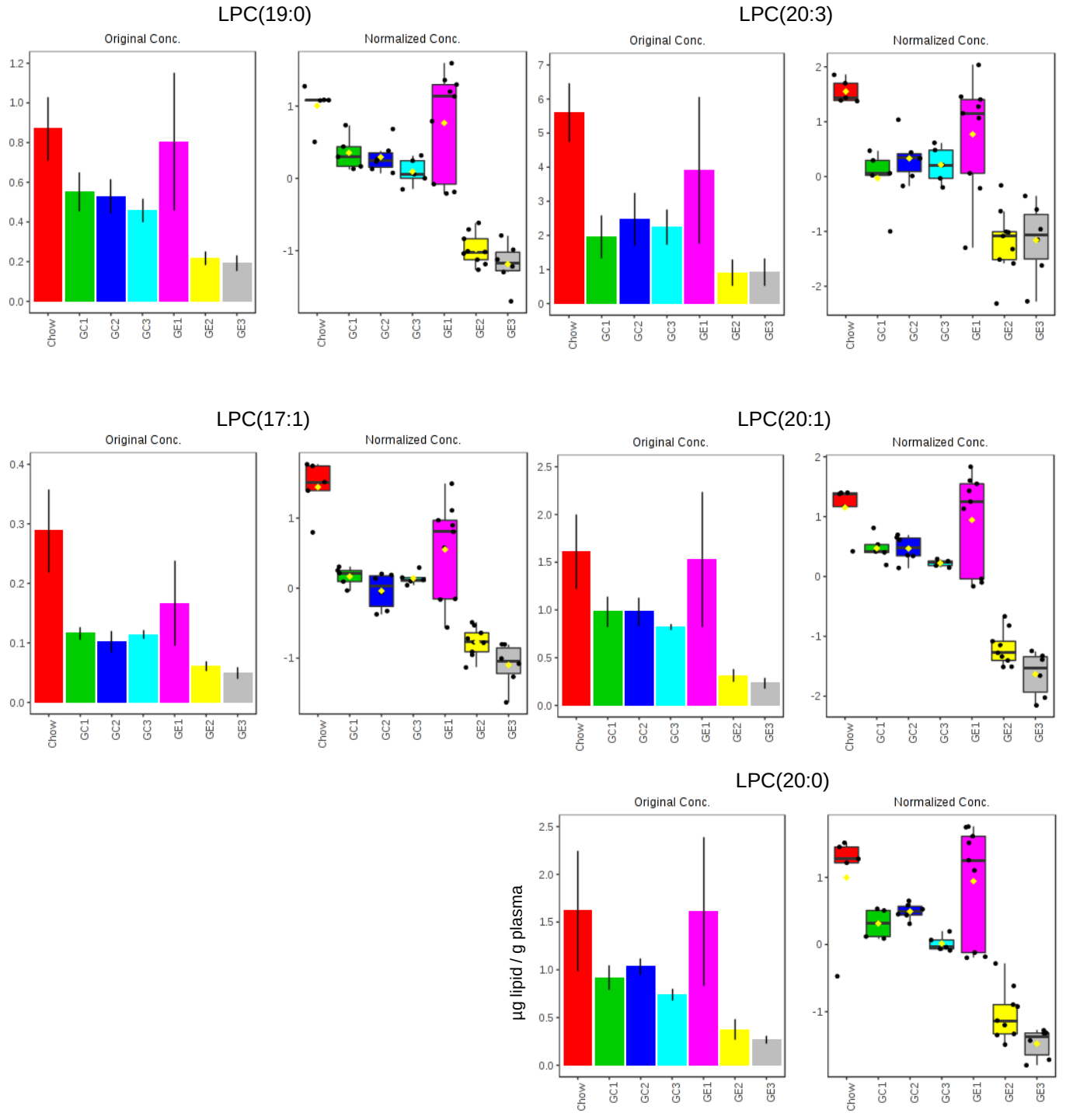


Figure S4A: Lysophosphatidylcholine species in plasma that differed most from pair-fed controls. Significance was determined between all time points and pair-fed controls according to an FDR corrected (Hochberg) ANOVA with a Tukey *post-hoc* test (p-value < 0.05). Lipids have a significant decrease between week 2 and 5, and are significantly different than controls for week 4 and 5. Further detailed descriptions are shown on page/slide 5.

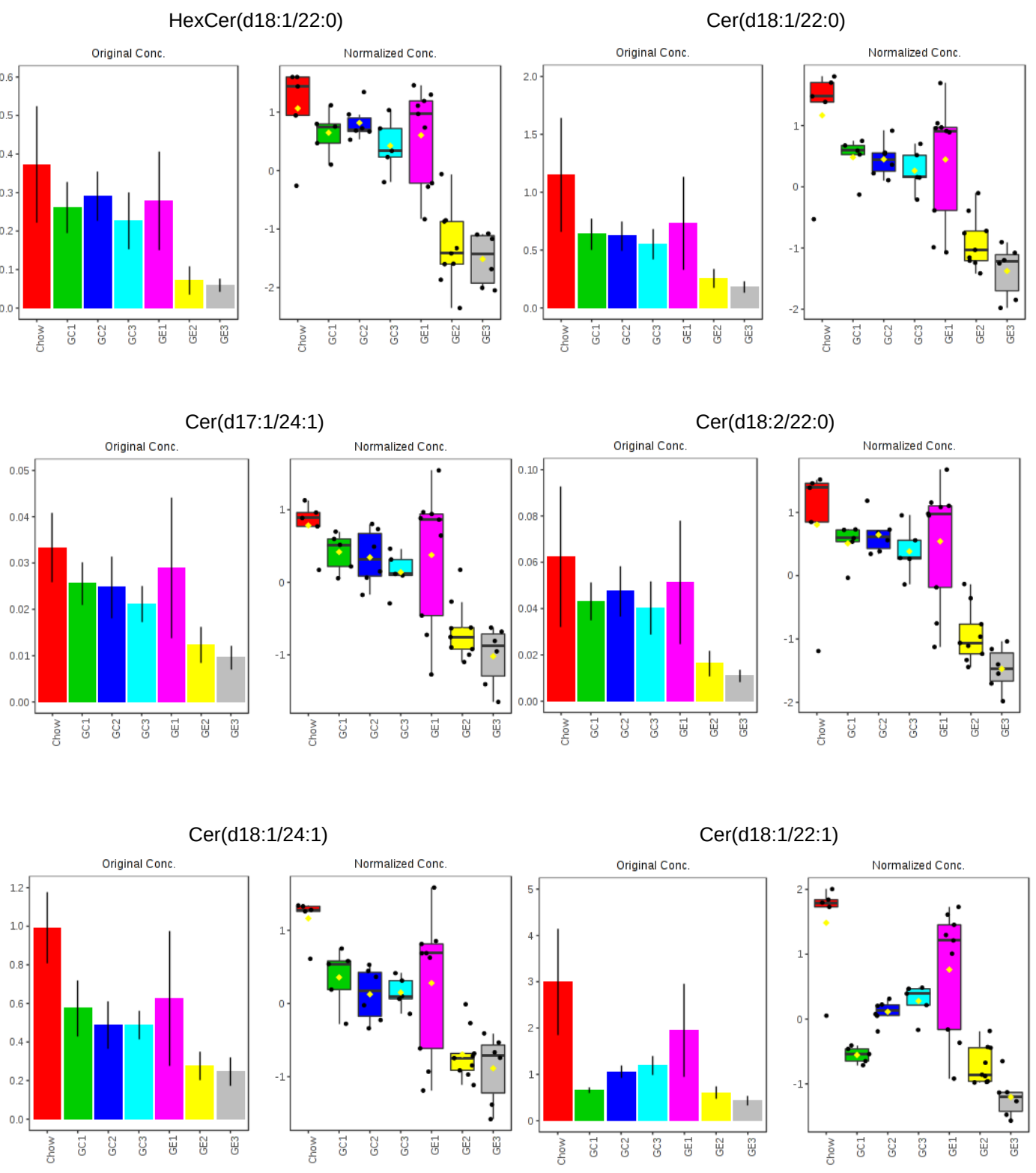


Figure S4B: The most significant sphingolipid species in plasma according to ANOVA FDR corrected p-value. Lipids have a significant decrease between week 2 and 5, and are significantly different than controls for week 4 and 5. SEE FIG. S4A LEGEND COMMENTS.

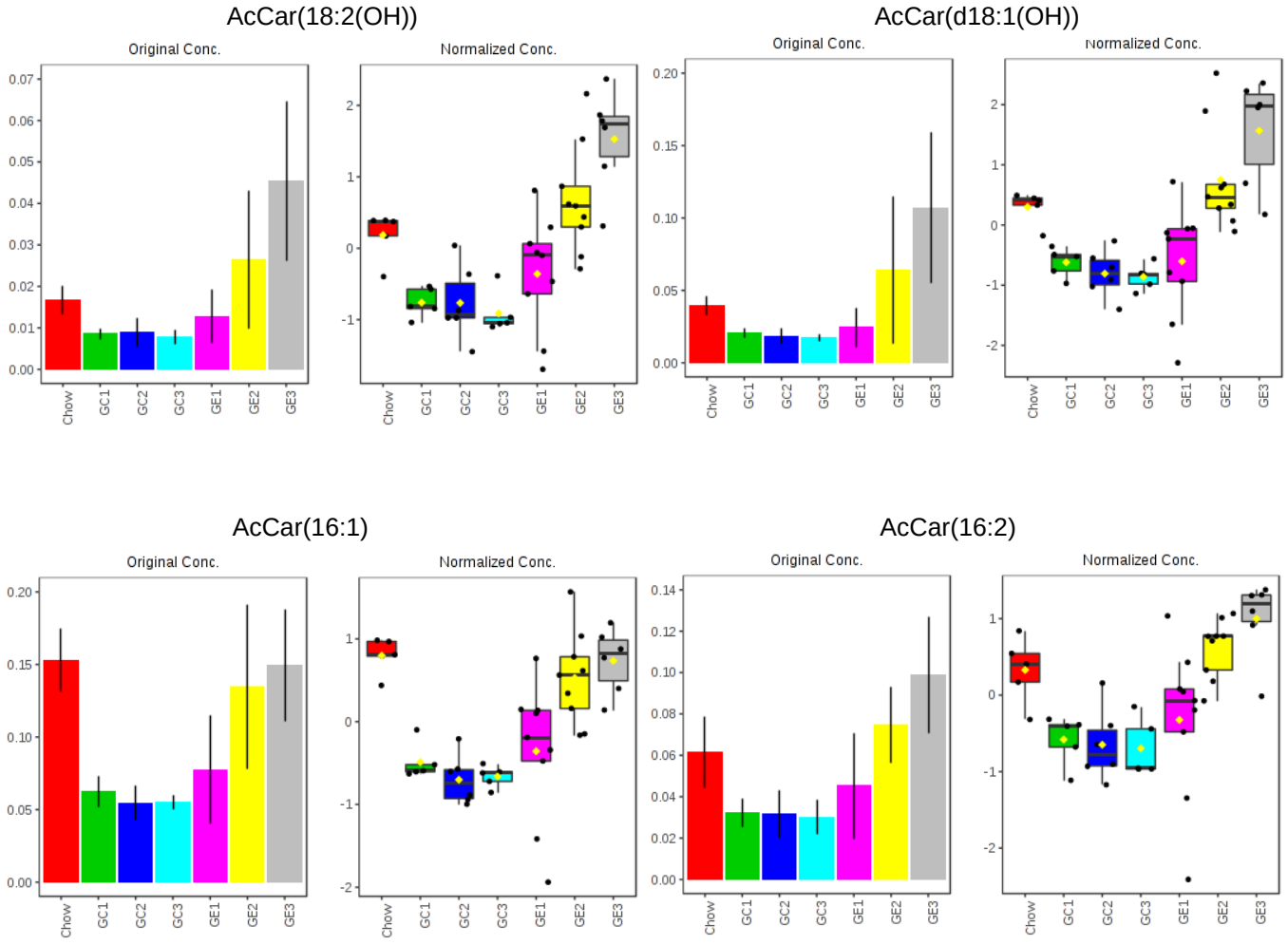


Figure S4C: The most significant acylcarnitine species in plasma according to ANOVA FDR corrected p-value. Lipids have a significant decrease between week 2 and 5, and are significantly different than controls for week 4 and 5. SEE FIG. S4A LEGEND COMMENTS.

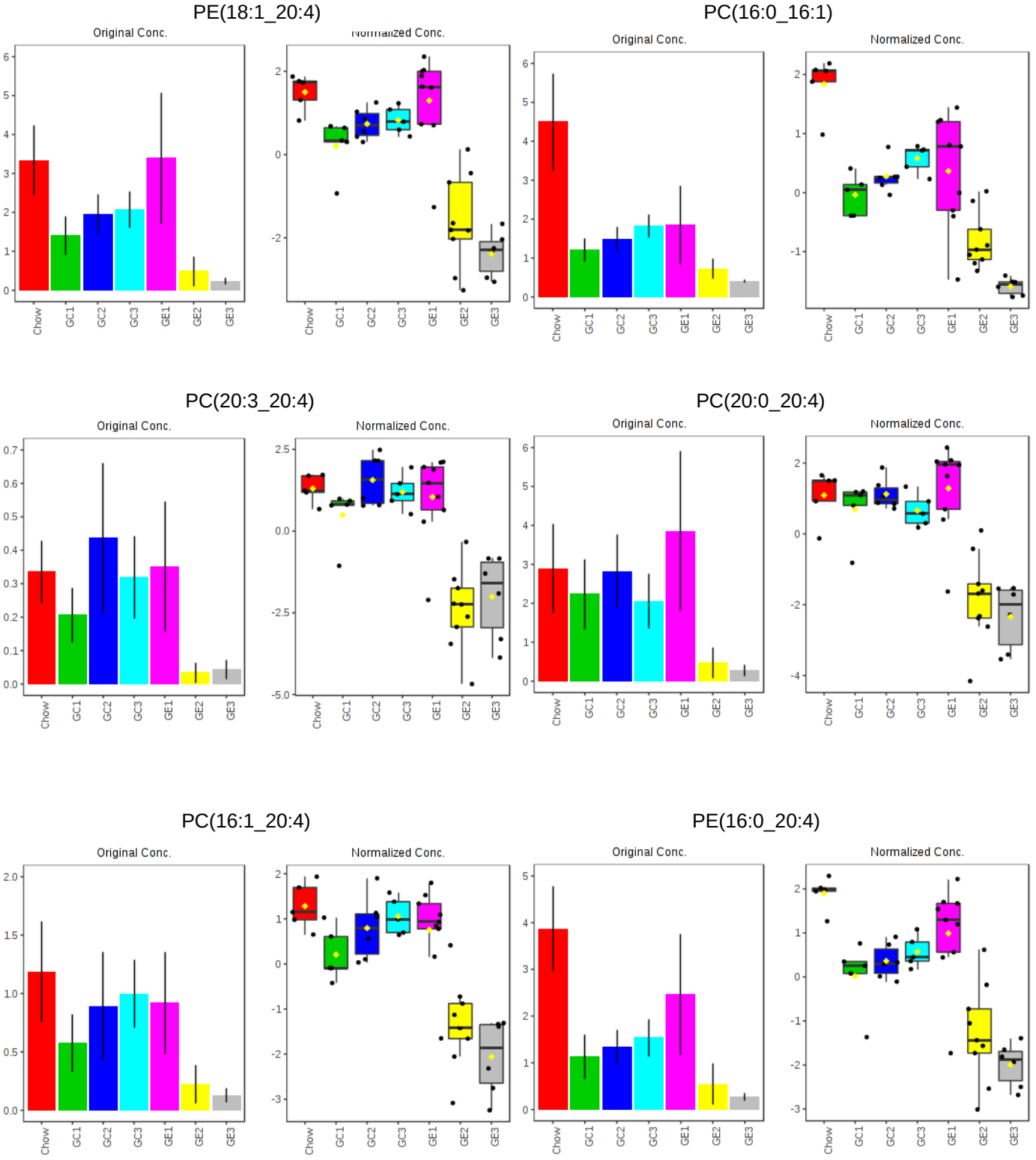


Figure S4D: The most significant glycerophospholipid species in plasma according to ANOVA FDR corrected p-value. Note that all but one of the species contains arachidonic acid (20:4). Lipids have a significant decrease between week 2 and 5, and are significantly different than controls for week 4 and 5. SEE FIG. S4A LEGEND COMMENTS.

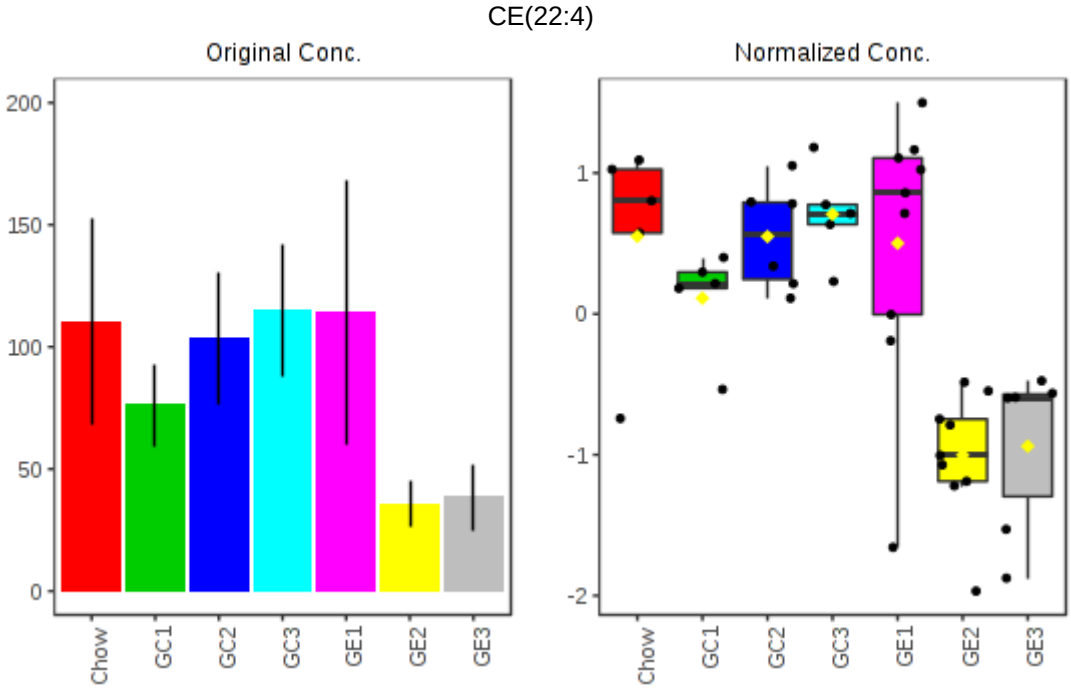


Figure S4E: The most significant CE in plasma according to ANOVA FDR corrected p-value. This lipid has a significant decrease between week 2 and 5, and are significantly different than controls for week 4 and 5. SEE FIG. S4A LEGEND COMMENTS.

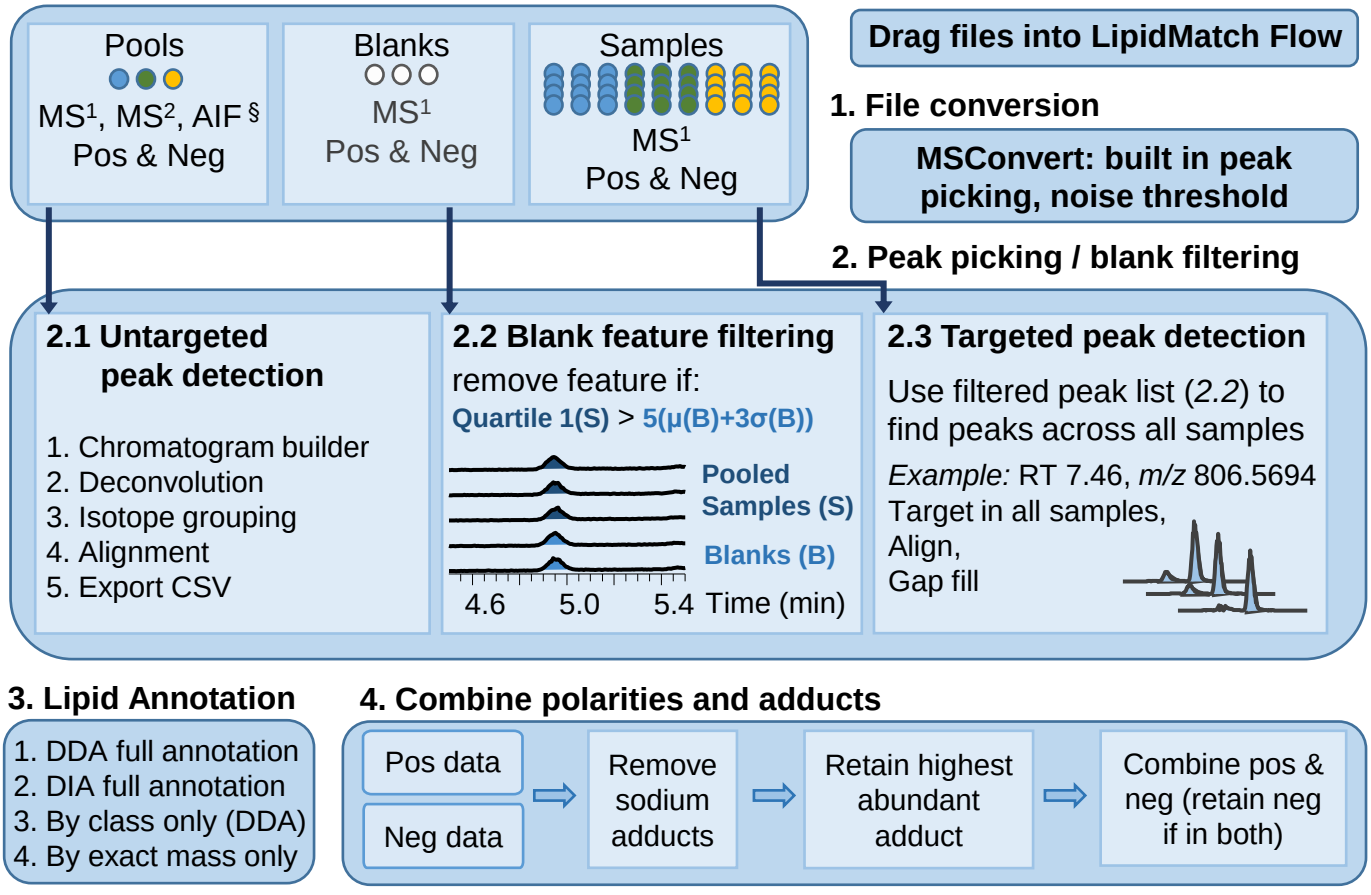


Figure S5: *Data-acquisition workflow and LipidMatch Flow data-processing workflow.* After dragging files into LipidMatch Flow, all further steps are automated.
Acronyms are defined as follows - RT: retention time, MS^2: tandem mass spectrometry, MS^1: full-scan data, Pos: positive ion mode, Neg: negative ion mode, *m/z*: mass to charge ratio, μ: average, and σ: standard deviation.
§ Data-independent analysis is only supported for Thermo's all-ion fragmentation (AIF). Otherwise, the software currently supports Agilent, Sciex, and Thermo targeted, data-dependent and full scan file formats.

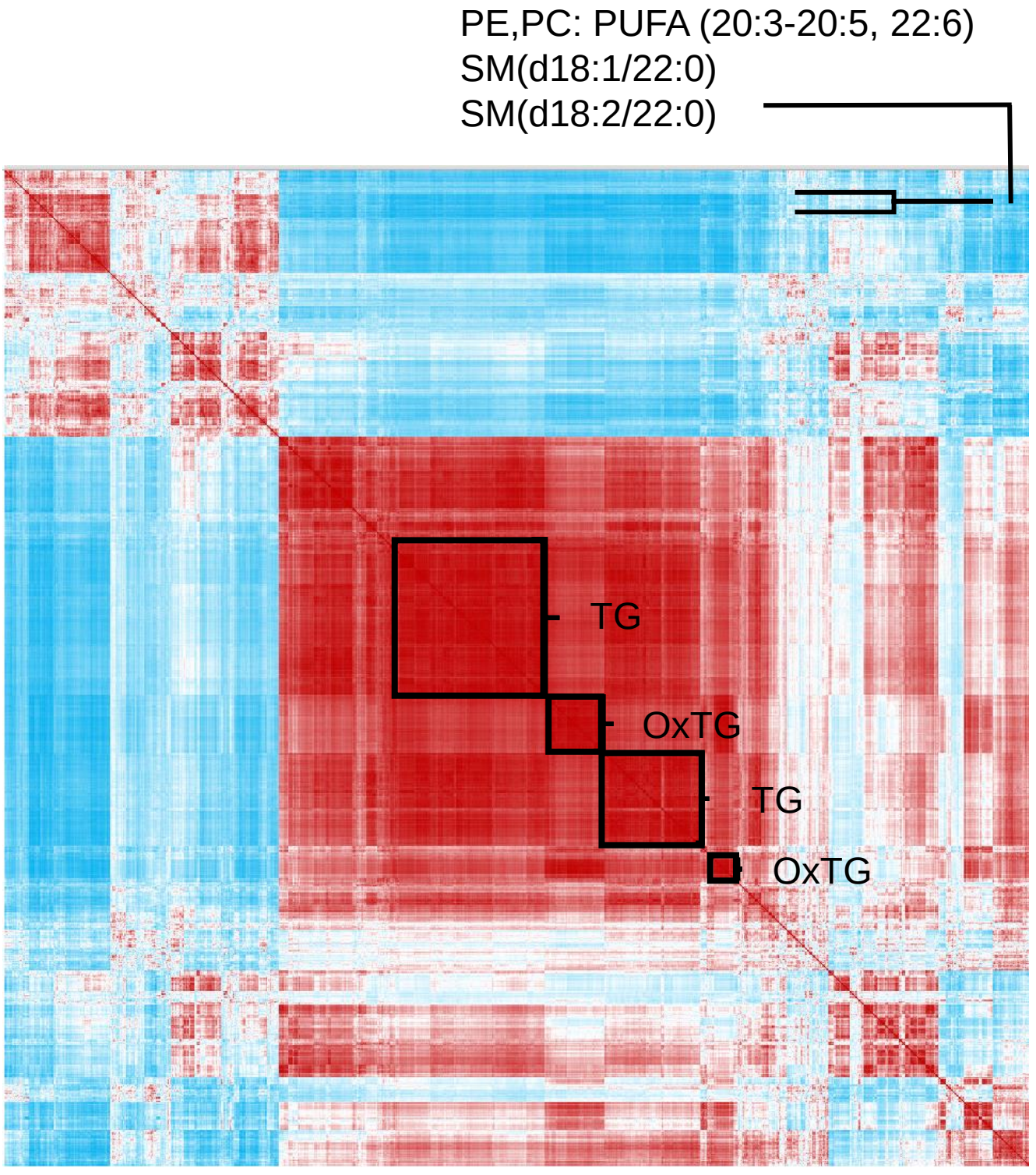


Figure S6: Correlation plot, showing major clusters of co-correlating and anti-correlating clusters of lipids. Pearson's correlation was used.

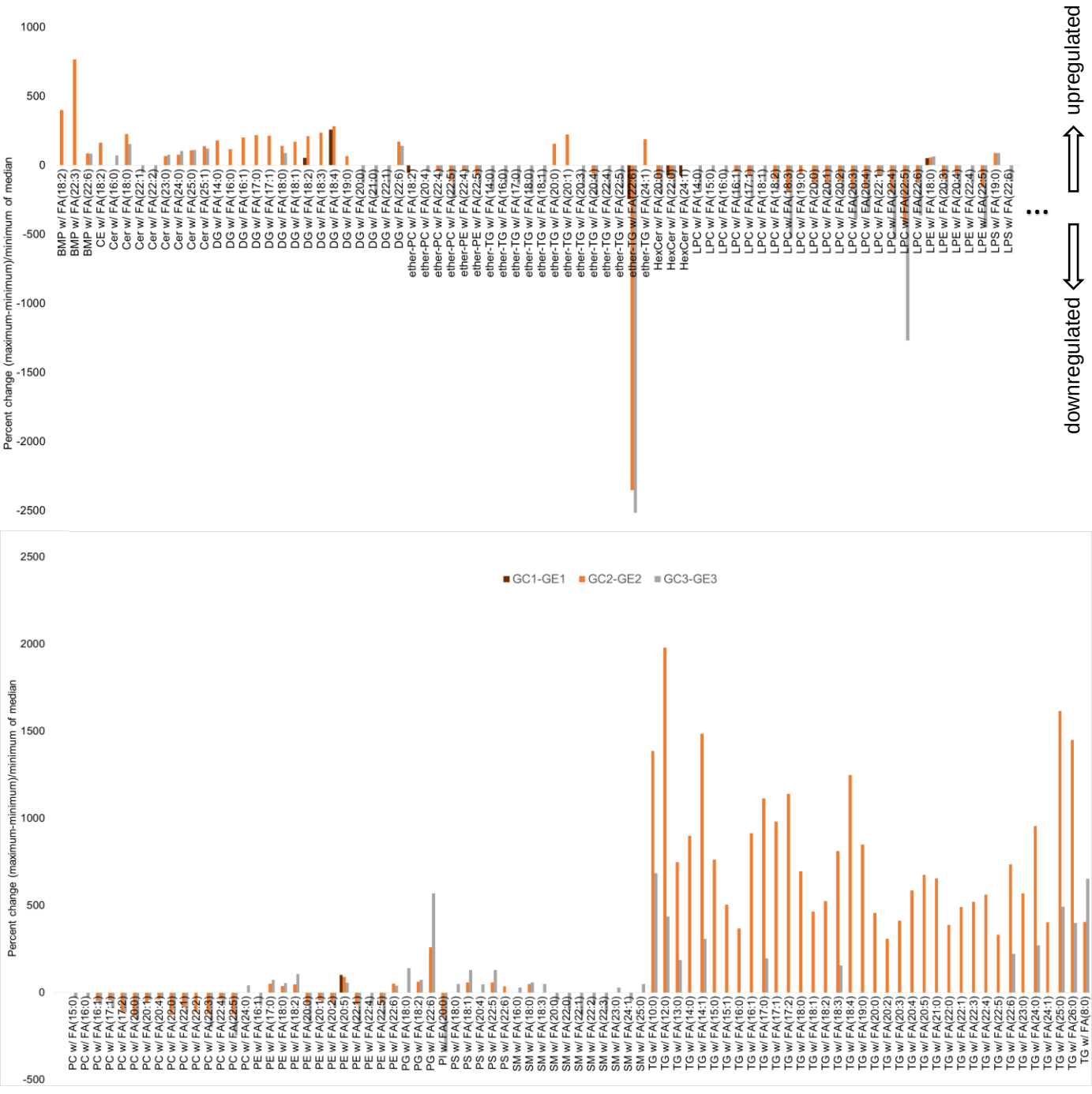
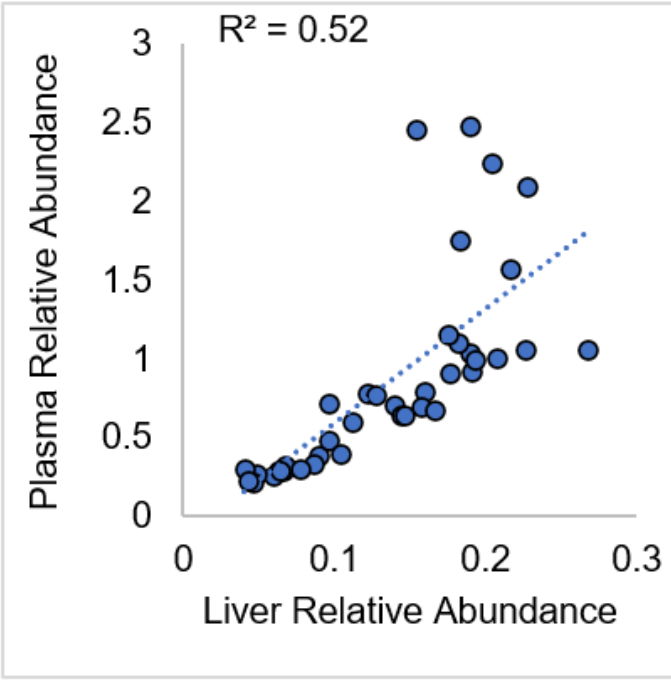


Figure S7: Percent change of the median fatty acid amount by lipid class in controls versus ethanol fed mice. Upregulated is higher in ethanol fed mice, whereas downregulated is lower. All class/fatty acid pairs shown were significant (< 0.05) based on an FDR corrected ANOVA and Tukey post-hoc test.

LPC(20:0)



PC(20:0_20:3)

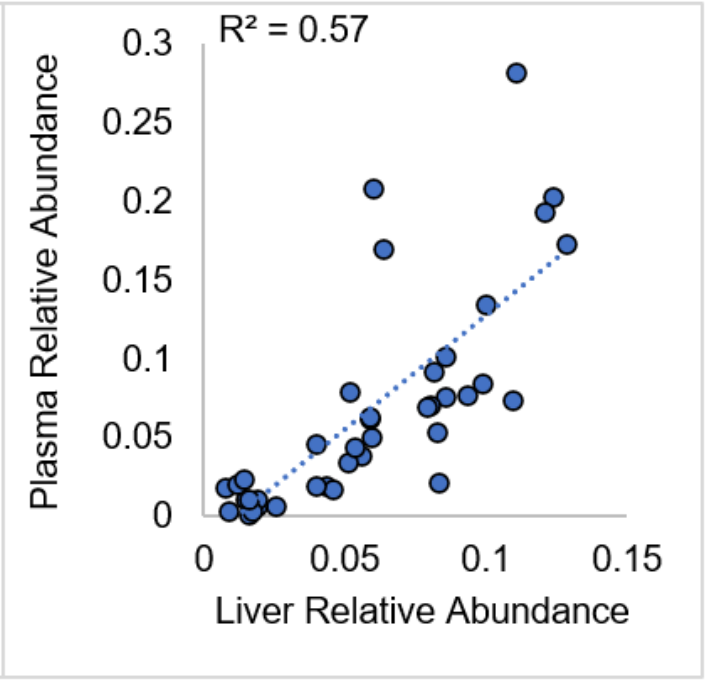
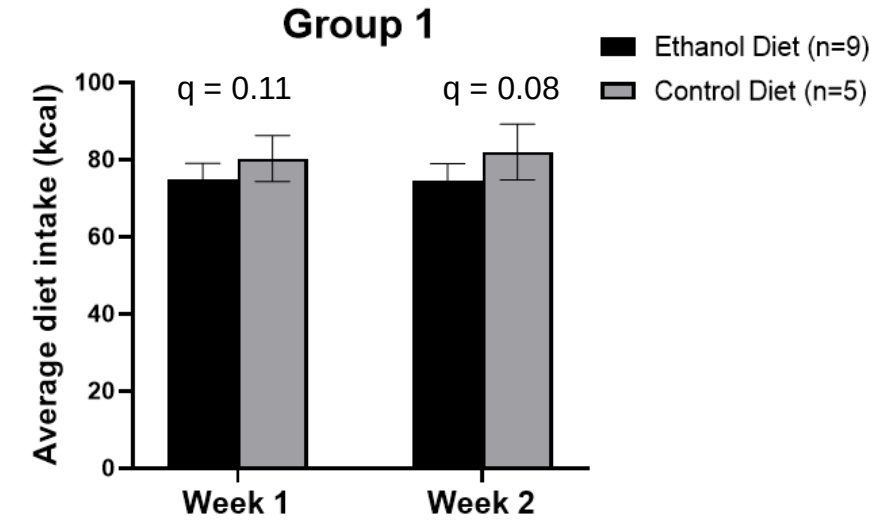
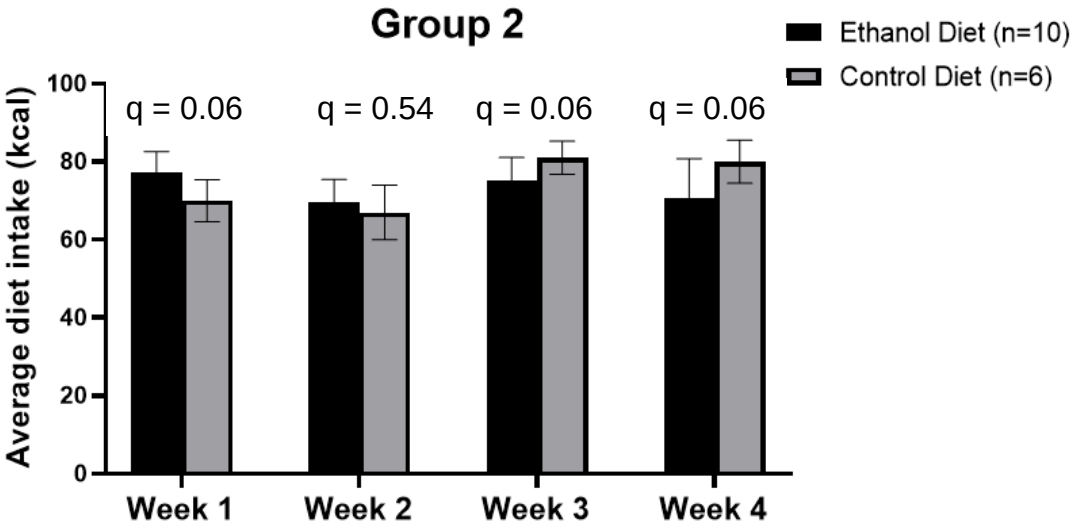


Figure S8: Two of the lipids with the strongest correlations between plasma and liver levels which also significantly changed in the 4th and 5th week of ethanol feeding.





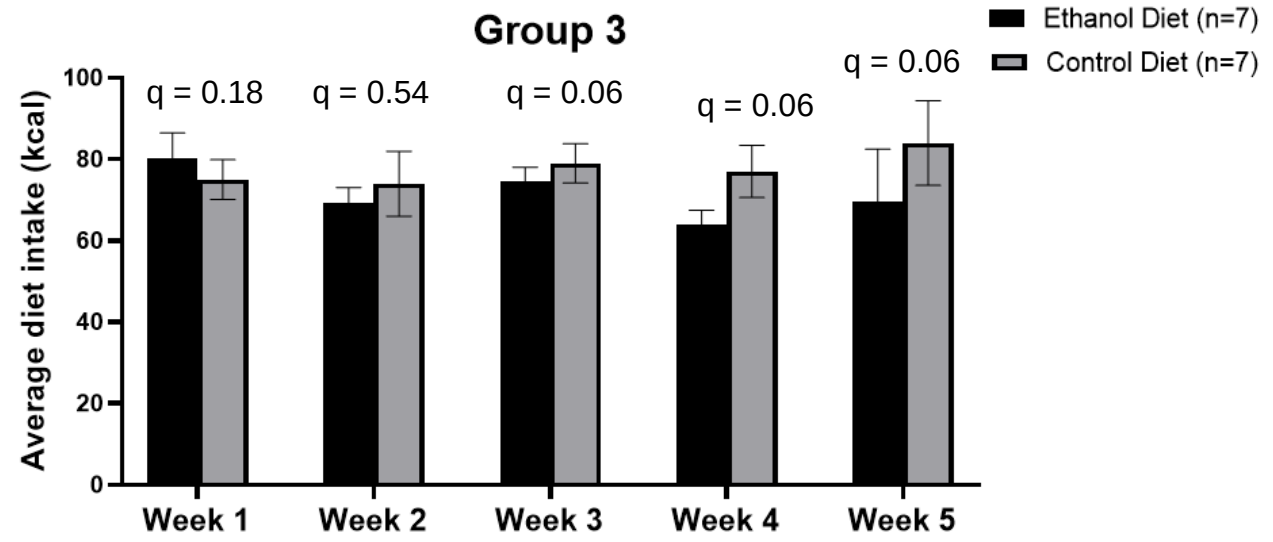


Figure S9: (Caption on next page)

Figure S9: (Previous page) Bar graph of recorded kilocalorie intake for mice across all groups and time points (average and standard deviation). No comparisons were significant using Hochberg correction (q-values) between ethanol-fed and control mice.